U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-297 493

Bioaccumulation of Heavy Metals by Littoral and Pelagic Marine Organisms

Moss Landing Marine Labs, CA

Prepared for

Environmental Research Lab, Narragansett, RI

Mar 79

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/3-79-038	2.
3. RECIPIENT'S ACCESSION NO. PB297493	
4. TITLE AND SUBTITLE BIOACCUMULATION OF HEAVY METALS BY LITTORAL AND PELAGIC MARINE ORGANISMS	5. REPORT DATE March 1979 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) John H. Martin	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Moss Landing Marine Laboratory Moss Landing, California 95039	10. PROGRAM ELEMENT NO. 1BA819 11. CONTRACT/GRANT NO. Grant 802350
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory - Narragansett, RI Office of Research and Development U.S. Environmental Protection Agency Narragansett, Rhode Island 02882	13. TYPE OF REPORT AND PERIOD COVERED Final 14. SPONSORING AGENCY CODE EPA/600/05
15. SUPPLEMENTARY NOTES -----	

16. ABSTRACT

Marine organisms appear to be useful indicators of heavy metal pollution in the marine environment. In order to test this concept, research was performed to determine the levels of heavy metals in selected indicator organisms. Several approaches were used. The first was to select intertidal invertebrates that are widely distributed and are readily accessible for collection. Tests with the limpet Acmaea scabra proved inconclusive, while those with the turban snail (Tegula funebralis) showed anthropogenic silver input. The experience gained from these studies indicated that serious problems could exist when using organsims as montiors. As a result, a study on pooling of individuals for monitoring studies was performed.

A second approach was to transplant oysters and mussels from clean to polluted environments in order to see if these organisms relfected ambient environmental levels. Significant increases in selected elements were observed in both bivalves and the general approach appears promising.

As is the case with many other pollutant studies, the general conclusion drawn from this study and the others mentioned above is that many marine organisms have high concentrations of heavy metals, but whether the metals are adversely affecting the organisms cannot be determined on the basis of measuring amounts alone.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Animal ecology Marine biology Metals	Sea Lions Copper toxicity Cadmium in plankton	6F 8A

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 65
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE PC A04/MF A01

EPA Form 2220-1 (9-73)

U.S. GOVERNMENT PRINTING OFFICE 1979-657-060/1635

United States
Environmental Protection
Agency

Environmental Research
Laboratory
Narragansett RI 02882

EPA-600/3-79-038
March 1979

Research and Development

PB 297493

EPA

Bioaccumulation of Heavy Metals by Littoral and Pelagic Marine Organisms

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/3-79-038
March 1979

BIOACCUMULATION OF HEAVY METALS BY LITTORAL AND PELAGIC MARINE ORGANISMS

by

John H. Martin
Moss Landing Marine Laboratories
Moss Landing, California 95039

Grant NO. R802350

Project Officer

Donald K. Phelps
Environmental Research Laboratory
Narragansett, Rhode Island 02882

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
NARRAGANSETT, RHODE ISLAND 02882

DISCLAIMER

This report has been reviewed by the Environmental Research Laboratory, Narragansett, U. S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U. S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The Environmental Research Laboratory of the U. S. Environmental Protection Agency is located on the shore of Narragansett Bay, Rhode Island. In order to assure the protection of marine resources, the laboratory is charged with providing a scientifically sound basis for Agency decisions on the environmental safety of various uses of marine systems. To a great extent, this requires research on the tolerance of marine organisms and their life stages as well as of ecosystems to many forms of pollution stress. In addition, a knowledge of pollutant transport and fate is needed.

This report describes a three-year study to measure concentrations of various metals by atomic absorption spectrophotometry in important littoral and pelagic marine organisms.

ABSTRACT

Marine organisms appear to be useful indicators of heavy metal pollution in the marine environment. In order to test this concept, research was performed to determine the levels of heavy metals in selected indicator organisms. Several approaches were used. The first was to select intertidal invertebrates that are widely distributed and are readily accessible for collection. Tests with the limpet Acmaea scabra proved inconclusive, while those with the turban snail (Tegula funebralis) showed anthropogenic silver input. The experience gained from these studies indicated that serious problems could exist when using organisms as monitors. As a result, a study on pooling of individuals for monitoring studies was performed and the results are reported here.

A second approach was to transplant oysters and mussles from clean to polluted environments in order to see if these organisms reflected ambient environmental levels. Significant increases in selected elements were observed in both bivalves and the general approach appears promising.

In addition to the monitoring research, we also studied several potential heavy metal problems. For example, we studied premature pupping in the California sea lion (Zalophus californianus) and found that normal mothers had molar ratios of 1 Hg:1 Se:1 Br, while the mothers having premature pups had Hg:Se ratios near unity, but Br concentrations were always depressed in these individuals. Whether these findings are directly related to premature pupping was impossible to ascertain. We also report that squid (Loligo opalescens) have extermely high Cu levels in their livers (up to 15 mg/g dry) and that the Cu is highly correlated with Ag. A study was also performed on cadmium in sea otters (Enhydra lutris). These mammals eat invertebrates almost exclusively; as a result, older individuals accumulate large amounts of this element especially in the kidney (up to 960 ug Cd/g dry weight). The relationship between Cd and other elements was also studied and the results are discussed.

Elevated Cd levels were also found in a study of plankton collected from Baja California waters. Subsequent research has revealed that the Cd is of natural origin and not from anthropogenic sources. As is the case with many other pollutant studies, the general conclusion drawn from this study and the others mentioned above is that many marine organisms have high concentrations of heavy metals, but whether the metals are adversely affecting the organisms cannot be determined on the basis of measuring amounts alone.

TABLE OF CONTENTS

Disclaimer ii

Foreword iii

Abstract iv

Section 1: Introduction 1

Section 2: The Use of Organisms as Pollutant Monitors 3

Section 3: General Studies on Metals in Marine Organisms 25

A. Premature Pupping in California Sea Lions 25

B. Silver, Cadmium, Cooper, Zinc and Iron in Squid Livers 27

C. Abalone Copper Toxicity Experiments 28

D. Cadmium in Plankton: Elevated Levels off Baja, California . . . 28

E. Cadmium in Sea Otters 30

Section 4: Methods 49

Publications and Theses Based on Research Supported by EPA Grant # R 802350 51

References

SECTION 1
INTRODUCTION

The toxicity of heavy metals has been the subject of a vast amount of research, in large part because of the threat posed by these elements to human health. For example, several disastrous cases of mass poisonings by mercury compounds have occurred in recent years; e.g., between 1953 and 1960, 121 cases of Hg poisoning were recorded in Minamata, Japan; 46 people died. In Iraq, mass Hg epidemics occurred in 1956 (100 cases; 14 deaths), in 1960 (1,000 cases) and in 1972 (6,530 cases; 459 deaths). (See Eyl, 1971; Bakir, et al, 1973.) Several other heavy metals are also toxic enough to represent human health hazards. Cadmium-induced decalcification of the skeleton (Itai Itai disease) has taken 56 lives in Japan (Nilsson, 1970); human lead poisoning has been known since antiquity (Patterson, 1965).

In addition to the threat to man himself, agricultural scientists have long been concerned with the toxicity of certain trace elements to valuable domestic animals. This is best exemplified by selenium, an element whose toxic effects were apparently first described in 1295 by Marco Polo during his travels through China (Trelease and Beath, 1949). This element is concentrated by certain plant species; herbivores grazing on these plants exhibit symptoms commonly called "alkali disease" and "blind staggers". These conditions can lead to mass mortalities; i.e., in 1906 and 1908, 15,000 sheep died of selenium poisoning in the state of Wyoming (Trelease and Beath, 1949).

Mass mortalities caused by heavy metals have also been observed in the marine environment. Approximately 2,000 abalone (Haliotis rufescens, H. cracherodii) died from copper poisoning when a power plant cooling system was tested (Martin, et al, 1977). The copper had leached out of copper-nickel tubing into sea water which had been standing in the system for several weeks. When the water was released, it contained approximately 1,899 ppb Cu, a concentration that resulted in the deaths of almost all of the abalone living in the immediate discharge area.

However, in comparison to the terrestrial environment, instances of this nature rarely have been reported in the marine environment. Nevertheless, research efforts on metals in the sea have been increasing in recent years because of a series of interrelated events. The well-publicized Minamata Bay disaster was caused by the consumption of marine food items that were contaminated with large amounts of methyl mercury. A few years later, tremendous advances in analytical capabilities for trace elements were being made, including the development of a very sensitive technique for Hg (Hatch and Ott, 1968). This led to the finding that important marine food items such as tuna and swordfish had relatively large quantities of Hg. In conjunction with these events, man was also becoming increasingly concerned not only with the quality of food items from the sea, but also with the quality of the marine ecosystem

itself. The combination of these developments resulted in a greatly increased emphasis on heavy metal research in the marine environment.

In order to learn more about these processes, we began a study on the bioaccumulation of heavy metals by littoral and pelagic marine organisms in January 1973. Most of our results have been published in the open literature and repeating these papers' contents in the text is not warranted. Abstracts of the papers are included in the sections that follow. In most cases, reprints can be obtained by writing the author. We will comment on the papers and discuss them in light of additional information made available after various portions of this research were published.

SECTION 2
THE USE OF ORGANISMS AS POLLUTANT MONITORS

The use of organisms as monitors of various pollutants is attractive for several reasons: Selected sessil organisms will continuously "sample" pollutants in their environment and thus smooth out large-scale fluctuations such as pulse input associated with some outfalls. They will also be subjected to materials from non-discrete point sources, which would be missed in human monitoring studies. In most cases, organisms will concentrate selected metals both from food sources and directly from water. Since some organisms concentrate large amounts of a given metal, the chances for sample contamination are minimized. In addition, it is easier and less costly to analyze biological tissues than water samples.

Organisms selected as biological monitors should have a wide range, occur in large quantities, be easily collectable from shore (eliminating the need for boats and diving equipment), be easily identified, have a simple life history and, in order to eliminate complex food web relationships, should also be of low trophic level. In addition to these biological factors, a monitor organism should, of course, concentrate metals of interest, preferably in the whole organism, rather than in a specific organ which can necessitate tedious dissections. Chances for sample contamination are also reduced when dissections can be avoided.

The following abstracts represent our research on the use of organisms as pollutant monitors:

Flegal, A.R. and J.H. Martin, 1977. Contamination of biological samples by ingested sediment. Mar. Poll. Bull. 8:90-92.
Abstract: An inorganic residue, presumed to be ingested sediment, was found in the rocky intertidal gastropods Tegula funebralis and Acmaea scabra and the estuarine copepods Acartia tonsa and A. clausi. When expressed as a percentage of the sample weight, this residue fraction often correlated significantly with the elemental concentrations measured in the organisms.

Flegal, A.R. Trace element concentrations of the rough limpet, Acmaea scabra, in California. (Accepted by Bulletin of Environmental Contamination and Toxicology).
Abstract: The trace element (Ag, Cd, Cu, Fe, Mn, Ni, Zn) concentrations of the rough limpet, Acmaea scabra, were determined at twelve locations along the California coastline. The mean silver concentration of the organisms was highest at Point laJolla, but no elemental concentration exhibited measurable geographic differences. Simple linear correlation coefficients and multiple analysis of variance statistics indicated a general independence of the elemental concentrations from each other and from other biological and geographic variables in the total sample.

Flegal, A.R. The geographic variation of silver in the black turban snail, Tegula funebralis.

Abstract: The geographic variation of silver concentrations in the black turban snail, Tegula funebralis, collected from the California-Mexico coastline indicates an anthropogenic influence. The relative variation in this species is also consistent with those reported for other organisms.

The experience that we gained in the above studies indicated that serious problems existed in attempting to use biological organisms as monitors.

For example, animals such as Tegula were very small and detection limits were pushed to their maximum. Sediment often occurred in association with the sample, which, in some cases, invalidated the findings for some elements. Tremendous variability between individuals was observed, even when the samples were collected in the same area on the same day. It was apparent that improvements could and should be made.

As is the case with almost all research, the state-of-the-art is continually improving in trace metal analyses. This is especially true since the completion of our studies. In recent years, the use of graphite furnace atomizers has decreased detection limits to the point where very small animals can now be analyzed successfully. This opens up a whole realm of species that were previously too small to serve as successful monitors. With the improved detection limits, analytical variability between samples will decrease markedly, provided proper corrections are made for the severe matrix problems that almost always are associated with the use of furnaces.

Nevertheless, graphite furnaces do nothing to improve variability inherent within the organisms themselves. Thus, the fact remains that large numbers of individuals must be analyzed in order to obtain statistically valid differences between selected areas. This is exemplified by the data in Tables 1 and 2.

Twenty-eight mussels (Mytilus edulis) were collected in San Francisco Bay for comparison with thirty individuals collected at Moss Landing, California. The data were then tested to determine the minimum number of replicates needed to determine valid differences for each element. Very few would be needed for Pb. The means and standard deviations are so different that only two individuals from each area would be required. However, in the case of Cd, over 120 individuals would have to be analyzed from each site in order to detect a significant difference. Clearly, more than 240 analyses would be unfeasible. This would be especially true if the analyses were performed using a graphite furnace. This is an extremely time-consuming technique, especially when the method of standard additions has to be used.

For these reasons, we have begun investigating the feasibility of pooling samples in order to obtain significant data with a minimal number of analyses. The following discussion was developed by George Knauer, Mike Gordon and Ann Hurley of our laboratories.

In order to establish baseline levels of trace metal concentrations in a given population, the first step must include estimates of the parameters μ (true population mean) and σ^2 (true population variance). Since it is not possible to measure all individuals in a population, it becomes necessary to sample a portion of the population of interest in an attempt to estimate the

TABLE 1: Concentrations of trace elements observed in mussels (*Mytilus edulis*) collected in an unpolluted environment (Moss Landing, California)

µg/g Dry Weight

Length (mm)	Pb	Cd	Cu	Zn	Mn	Fe	Al	Ni	Ag	Cr
57	.38	6.9	17.5	46	4.5	230	120	1.0	.22	1.0
57	.27	9.9	12.4	64	3.3	200	100	.9	.12	1.0
56	.90	13.3	20.3	302	7.5	690	480	3.4	.13	3.0
56	.31	11.0	20.9	102	5.3	370	200	2.1	.19	2.1
49	.51	14.3	25.2	126	8.3	570	400	3.0	.23	1.4
52	.58	11.5	19.2	279	6.3	480	340	1.9	.06	1.7
50	.23	13.7	20.3	122	7.0	410	280	2.1	.19	1.9
51	.16	9.7	20.4	142	7.2	510	360	2.8	.19	2.4
52	.32	3.8	14.9	114	4.4	190	120	1.0	.11	.7
47	.12	10.7	24.2	105	9.1	670	460	3.0	.21	2.4
46	.48	12.8	15.4	232	4.7	260	110	1.1	.13	1.1
60	.01	9.4	17.3	98	2.8	220	10	.9	.21	1.2
48	.16	9.8	15.2	47	4.7	260	120	1.0	.18	1.0
43	.36	6.4	23.1	138	11.4	810	590	3.7	.20	3.6
45	.16	8.0	19.6	280	5.2	430	240	1.8	.20	1.8
54	.27	17.0	19.5	195	6.8	400	260	2.0	.16	1.2
50	.29	9.9	17.4	61	5.7	360	230	1.1	.20	2.0
46	.17	14.0	13.7	75	3.8	210	90	.8	.15	1.9
48	.27	7.0	16.4	118	5.6	370	300	1.1	.16	1.7
52	.63	9.2	23.1	101	9.0	750	560	3.1	.16	2.0
49	.84	14.0	22.5	266	8.1	440	290	1.7	.25	2.1
55	.49	23.9	29.2	162	12.4	920	640	3.2	.31	3.4
52	.26	15.8	15.7	170	5.0	340	180	1.1	.14	1.5
48	.39	17.2	23.8	166	8.4	720	480	3.3	.19	2.4
56	.71	12.8	13.8	98	4.2	300	160	1.4	.13	.6
54	.23	12.5	22.2	240	6.5	300	180	.4	.19	1.1
59	.17	13.8	19.5	499	6.7	410	200	2.0	.24	2.4
50	.29	5.3	18.4	138	6.0	300	160	1.7	.10	1.2
47	.32	5.2	12.6	159	7.5	490	360	1.6	.20	1.9
44	.45	6.4	16.0	193	5.9	340	220	.7	.11	1.3
Mean	.36	11.2	18.8	161	6.6	430	270	1.8	.18	1.7
Std Dev	.21	4.3	6.0	96	2.7	200	160	1.0	.05	.8

TABLE 2: Concentrations of trace elements observed in mussels (*Mytilus edulis*) collected in a polluted environment (San Francisco Bay)

Length (mm)	µg/g Dry Weight									
	Pb	Cd	Cu	Zn	Mn	Fe	Al	Ni	Ag	Cr
47	2.05	3.2	19.2	156	97.2	360	340	6.4	.22	2.1
54	2.17	10.0	32.4	570	131.0	840	770	10.4	2.33	3.2
56	4.98	11.4	40.4	437	164.8	520	440	9.6	.56	2.2
48	3.19	7.8	45.2	587	126.4	670	560	14.4	.38	3.2
48	2.71	12.5	29.7	380	22.1	140	40	6.7	1.01	.9
61	2.59	11.8	23.4	182	63.2	400	290	5.0	.19	1.0
43	4.23	8.9	39.4	565	150.6	700	620	10.1	.64	3.0
55	1.53	23.2	35.9	184	124.0	470	390	8.5	1.32	2.7
59	.16	15.7	23.0	110	54.8	350	280	8.0	.18	1.5
53	2.92	10.4	22.1	136	48.8	340	280	6.1	.20	2.0
58	.44	11.2	21.9	62	41.7	290	230	7.8	.13	1.7
47	9.40	11.0	28.8	353	62.2	470	420	9.4	.49	2.3
65	.84	11.2	26.5	130	41.7	340	280	11.0	.21	2.1
46	2.66	7.7	33.0	382	86.4	500	390	11.0	.28	1.8
60	1.09	10.4	28.1	185	59.1	300	270	8.0	2.46	1.6
53	.39	19.7	27.9	136	14.6	150	120	11.7	.63	1.4
51	3.33	21.4	44.4	274	68.1	500	420	16.2	1.06	3.2
56	3.90	23.1	35.2	169	27.8	220	130	10.6	.28	2.2
60	.73	20.2	35.4	128	28.2	310	230	10.9	.31	1.4
59	.21	17.8	27.2	124	85.1	530	450	8.9	.67	2.2
50	1.86	10.4	25.0	93	71.4	410	340	7.0	.22	1.3
61	1.37	8.4	22.1	133	43.1	140	70	5.4	.33	.6
53	2.52	10.4	32.8	252	18.6	90	70	4.4	.30	.7
52	7.48	10.9	28.1	714	136.8	590	580	11.3	.28	2.2
48	7.36	13.0	28.2	472	115.7	420	350	7.6	.25	1.9
63	.72	9.5	31.8	165	66.5	390	300	10.8	.59	2.1
60	3.70	12.4	31.2	316	48.3	290	270	8.4	.24	1.8
53	4.36	13.4	33.2	497	87.9	520	500	6.6	1.15	2.4
Mean	2.82	12.8	30.4	281	74.3	400	340	9.0	.60	2.0
Std Dev	2.31	4.2	6.7	182	42.4	180	170	2.7	.59	.7

above parameters with the statistics $\bar{X}$ (estimated population mean) and σ^2 (estimated population variance). It is an accepted fact that the larger a random sample from a normally distributed population, the better the estimate of μ and σ^2 will be. It should be emphasized however, that each variable (i.e., Cu, Zn, etc.) has its own true mean and variance.

Clearly, our aim is to establish the best possible estimate of the true population mean and variance for metals of interest in a selected species with the expenditure of a reasonable amount of effort. As an illustration of the process that we intend to follow to estimate μ and σ^2, we have used the Cu data from analyses of samples of Mytilus edulis collected from Moss Landing in 1975 (Table 1). Cumulative means; e.g., $\bar{X}_1+X_2/2$, $X_1+X_2+X_3$, $X_1+\ldots X_{30}/30$, (Elliott, 1971), were plotted against numbers of individuals/mean to determine the number of individuals which must be analyzed to give a good estimate of μ and σ^2 (see Figure 1). Fluctuations about the calculated mean at n=30 (18.9 µg Cu/g dry wt.) become minimal around n=18. From this, it is logical to conclude that the analysis of eighteen individuals should give a reasonable estimate of μ. Note that Figure 1 clearly suggests that analysis of five to ten samples will not yield an adequate estimate of μ. Of course, this is for one element in one species. Similar tests would have to be performed for other elements and species.

The detection of a given difference between means will depend on the estimates of μ and σ^2 and upon the sampling design chosen. For example, use of the Cu results obtained from Mytilus can be used to arrive at a number of different sampling design schemes. Our best estimate of μ and σ^2 (standard deviation) using the Mytilus Cu data are 18.9 and 4.14, respectively. We have used these Mytilus Cu data to calculate the number of samples required to detect a statistically significant difference ($P < 0.05$) between two means which differ by 5 - 100%. The results are presented graphically in Figure 2 and in tabular form in Table 3. These data can be used to determine the number of individual analyses needed to detect some desired difference between means; e.g., 20%, or the number of individuals which can be pooled to yield a similar resolution. Note that one individual/pool is equivalent to the analysis of n individuals that would be necessary to achieve some desired difference between means. The advantage of pooling two or more individuals becomes apparent when the detection of small differences between means with a minimal amount of effort is desirable. It may be seen from Table 3 that, as the number of individuals/pool increases, the number of pooled samples decreases up to the level of 75% difference between means. At the 75% and 100% difference, three pools are needed regardless of the number of individuals/pool. Clearly, there are an infinite number of sampling designs which could be employed from these data; however, the choice of a single design depends upon many factors. These include the effort required to collect and dissect the organisms, the analytical time/sample and the total analytical load. For example, if it is desired to resolve a 20% difference between two means such as between 10 and 12 ppm Cu, we could analyze twenty-seven individuals (1 individual/pool, 27 pools), three individuals/pool with twelve pools or ten individuals/pool with four pools with respect to Cu in Mytilus. It should be made clear that, when individuals are pooled, more total organisms are re-

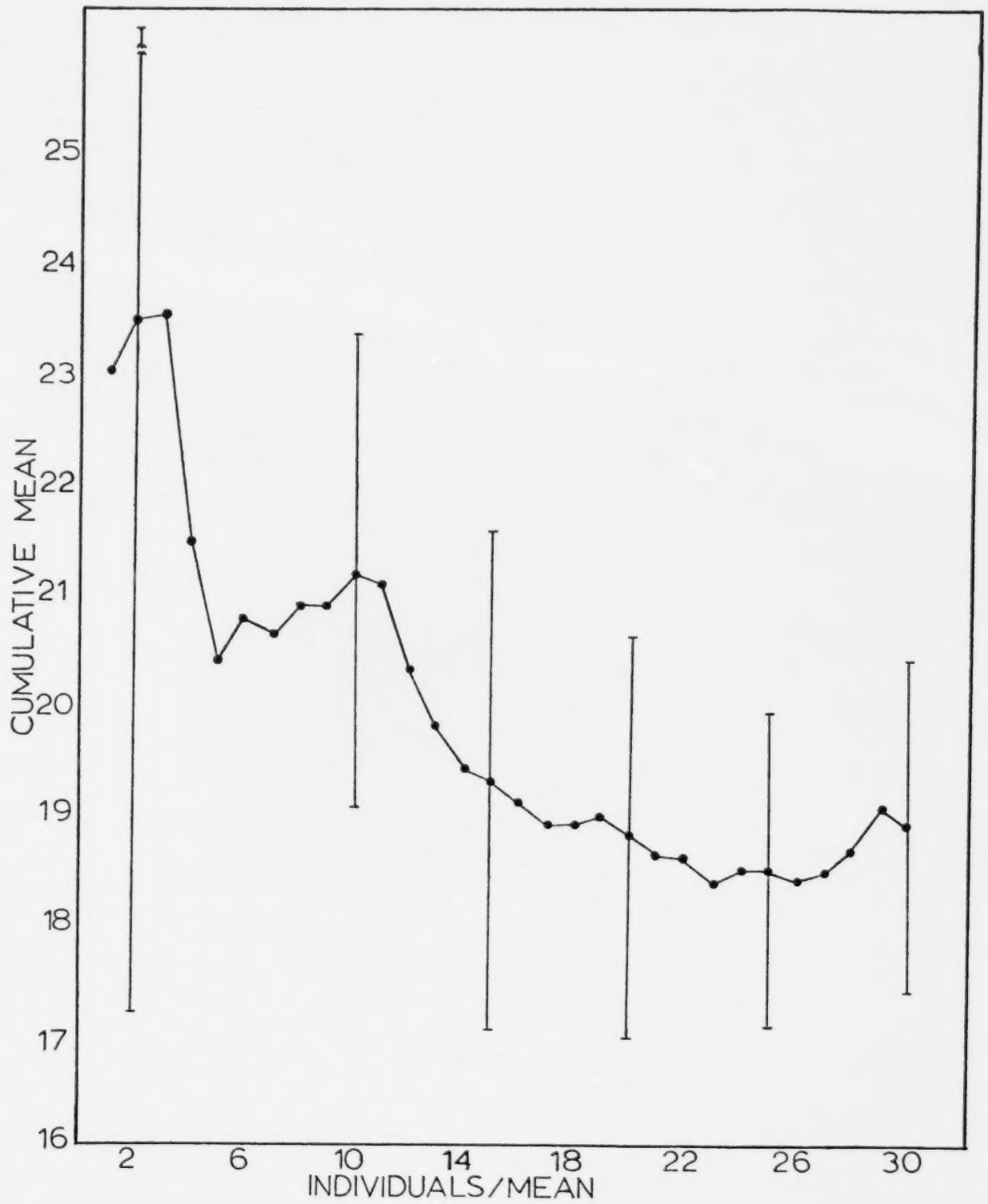

FIGURE 1: Cumulative Copper Means (With Selected 95% Confidence Intervals) from Analysis of n=30 Mytilus

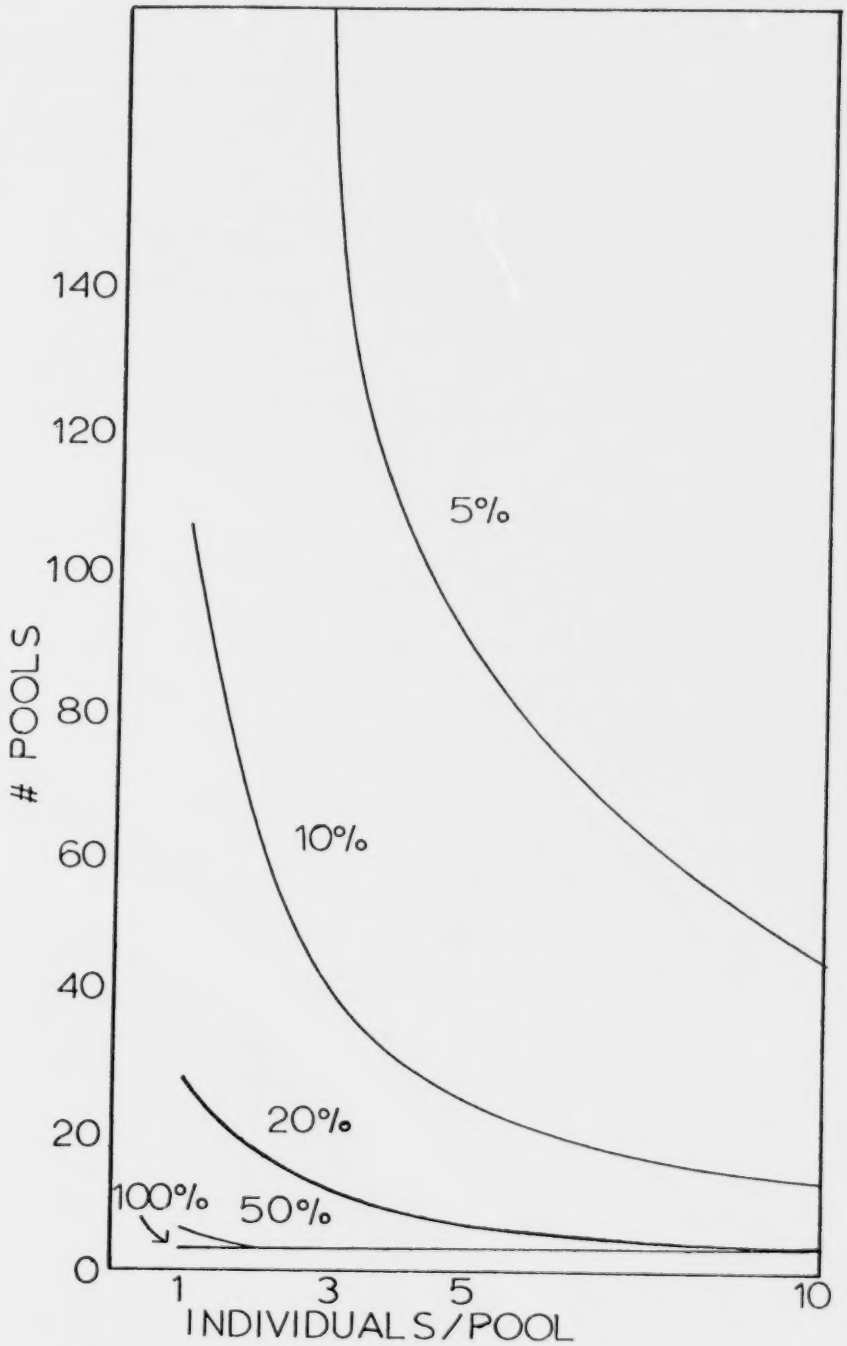

FIGURE 2: Plot Showing Number Pooled Mussel Samples (Ordinate) and Number Individuals/Pool (Abscissa) To Be 90% Certain (1-β, Type II Error) of Detecting 5, 10, 20, 50 or 100% Difference Between Two Copper Means at $P < 0.05$ Using t-Test

TABLE 3: Number of pools required to be 90% certain of detecting a significant difference ($P < .05$) between two means (Cu in Mytilus) differing by 5, 10, 20, 50, 75, 100%; $d' = (\mu_1 - \mu_2)/\sqrt{2}\sigma$ (Dixon and Massey, 1969)

Difference in Means (%)	Individuals/Pool	d'	Number Pools
5	1	.16	556
5	3	.28	146
5	5	.36	88
5	10	.51	44
10	1	.32	106
10	3	.53	40
10	5	.68	25
10	10	.97	13
20	1	.65	27
20	3	1.09	12
20	5	1.41	7
20	10	1.99	4
50	1	1.62	6
50	3	2.79	3
50	5	3.60	3
50	10	5.10	3
75	1	2.42	3
75	3	4.19	3
75	5	5.40	3
75	10	7.50	3
100	1	3.23	3
100	3	5.60	3
100	5	7.20	3
100	10	10.20	3

quired than when individual analyses are performed. In the above example, twenty-seven organisms were required when individuals are analyzed, but thirty-six organisms are required, i.e., three individuals/pool, twelve pools, to yield the same level of resolution for pooled samples. However, if there is no limit to the number of individuals available, then pooling may be desirable in that the analytical load can be reduced considerably.

The above discussion suggests that pooling of large numbers of individuals with consequent digestions and analyses of a few pools will enhance the chance for successful monitoring using organisms. However, this concept will have to be thoroughly tested using actual samples. We are participating in the "Mussel Watch" (National Marine Pollution Monitoring Program) in cooperation with Dr. Edward Goldberg of Scripps. The project is funded by EPA and involves the collection of mussels from the conterminous United States and subsequent analyses by selected investigators for various pollutants. This is a very prodigious undertaking. Time-saving procedures such as pooling may be particularly appropriate to large-scale studies such as these.

Another approach to using marine organisms as marine monitors is that of transplanting selected organisms to environments of interest and observing changes in metal concentrations with time. A manuscript using this approach is presented in its entirety. References mentioned are included in the report reference section.

BIOLOGICAL MONITORING OF TRACE METALS IN THE MARINE ENVIRONMENT WITH TRANSPLANTED OYSTERS AND MUSSELS

M.D. Stephenson
CALIFORNIA DEPARTMENT OF FISH AND GAME
Monterey, California 93940

R.M. Gordon
J.H. Martin
MOSS LANDING MARINE LABORATORIES
Moss Landing, California 95039

ABSTRACT

Oysters (Crassostrea gigas) and mussels (Mytilus edulis) were transplanted from a relatively unpolluted area to locations where trace metal monitoring was desired for a period of thirty days. These species were most effective in accumulating Ag, Cd, Cu and Ni. Significant differences between the control sites, Redwood City, Monterey sewage outfall and Elkhorn Slough, were observed. Transplanting is an effective method of detecting and monitoring trace metal inputs in marine waters.

INTRODUCTION

Trace metals in the marine environment have been monitored by a variety of techniques, including analyses of seawater, suspended particulates, sediments and biological material (Martin, et al, 1976; Bruland, et al, 1974; Preston, et al, 1972; Anderlini, et al, 1975). The analysis of biological material has proven valuable because organisms generally contain easily detectable concentrations of metals, are relatively easy to collect, are relatively inexpensive to analyze and can provide an integrated record of environmental trace metal concentrations over the lifespan of the organism. The establishment of monitoring baselines for trace metals in marine organisms, however, has sometimes proven difficult because of the high variability between individual organisms. Factors that contribute to this variability include:

1. Individual organisms may vary in metal content with size and age (Mackay, et al, 1975; Schulz-Baldes, 1973; Romeril, 1971). Organisms of the same size may not be found at all monitoring sites.

2. Many species are too small for analyses.

3. Many animals are covered by, or contain, sediment in their digestive systems (Flegal, 1978a; Flegal and Martin, 1977), which may contaminate the sample.

4. Individual tissues vary in metal concentrations. Whole organisms are analyzed instead of individual tissues. In this way, tissues that concentrate trace metals are combined with tissues that do not (or are contaminated), thus obscuring results.

5. Organisms may undergo seasonal changes in trace metal concentrations (Bryan, 1973; Phillips, 1976).

6. Intertidal organisms may vary in trace metal concentration in relation to the tidal heights at which they live (Phillips, 1977).

An approach using transplanted organisms such as oysters and mussels reduces the influence of many factors which contribute to high sample variability. The goal is not to establish a baseline, but to detect changes in trace metals over a given time period. Transplanting organisms has the following advantages over collecting resident organisms:

1. Large numbers of individuals are available either commercially or in natural beds.

2. Oysters can be aged in most cases and all organisms can be the same age.

3. There are few sediment contamination problems with these organisms.

4. Large enough individuals can be obtained so that individual tissues are easily dissected and analyzed.

5. The samples can be run inexpensively with flame atomic absorption spectrophotometry for most metals if large individuals are obtained.

6. The organisms can be easily transplanted to almost any site in the marine environment.

One transplantation design was evaluated in this study. Oysters (Crassostrea gigas) and mussels (Mytilus edulis) were collected from relatively unpolluted areas and transplanted to various locations. Uptake rates were determined and related to the environment to which they were transplanted.

METHODS

Oysters (C. gigas) were collected at Johnson's Oyster Company at Drake's Bay, California on May 17, 1975. They ranged from 9 to 14 cm in length. The gills of ten individuals were immediately dissected for later analysis. Groups of ten individuals were placed in nylon net bags (1/2-inch mesh) and suspended in the water column from a buoyed polypropylene line. Mussels (M. edulis) were collected from the west side of Tomales Bay on May 17, 1975 and treated in the same manner as the oysters.

Resident populations of oysters and mussels were analyzed to compare with the transplants and to determine the tissues to be used. Four and one-half-year-old C. gigas and seven-year-old C. virginica (originally transplanted to Redwood City as spat) were collected at Redwood City in April 1975. M. edulis were collected from native populations at Elkhorn Slough and Redwood City in April 1975.

The control areas, Drake's and Tomales Bays, are located approximately one hundred miles north of San Francisco and have no major anthropogenic discharges. Organisms were transplanted to the Port of Redwood City and Elkhorn Slough (enclosed bays), Monterey sewage outfall (semi-protected outer coast) and Granite Canyon (exposed outer coast). The Port of Redwood City pier is located about thirty miles south of San Francisco within San Francisco Bay. It was selected because it is within the San Francisco metropolitan area and is near to a major municipal sewage discharge as well as being near many industrial discharges and therefore should have maximum exposure to pollutants. Monterey sewage outfall is located approximately one-half mile east of the harbor at Monterey, California. It was selected because it is an area with a small municipal sewage discharge (2.2 mgd) and is without any major industrial discharges. Elkhorn Slough is located about one hundred miles south of San Francisco. It receives flow from the Salinas River, which, in turn, receives municipal sewage discharges from several small communities. The harbor at the mouth of the slough has

several hundred boats. In addition, there is a cooling water discharge from a large fossil fuel power plant. This area was selected because it is a relatively clean enclosed bay, in contrast to Redwood City.

Tissues from the resident populations of oysters and mussels were dissected from animals that had been frozen, while the gills of transplanted organisms were dissected fresh. The tissue was dried at 70 °C for 24 hours, dissolved with 70% quartz redistilled G. Frederik Smith redistilled HNO_3, charred at 350 °C and oxidized with 30% hydrogen peroxide (H_2O_2). The solution was diluted to 15 mls with 1% HNO_3 and analyzed by flame on a Perkin-Elmer Model 603 atomic absorption spectrophotometer. The deuterium arc background corrector was used to correct for non-atomic absorption. Cu and Ag analysis of M. edulis gill tissues required the use of the Perkin-Elmer HGA 2100 graphite furnace. These digestion and analysis techniques were shown to be accurate by the digestion and analysis of National Bureau of Standards orchard leaves and bovine livers.

Medians were used instead of means to compute accumulation rates, because medians are less affected by extreme values (Sokal and Rohlf, 1969). A non-parametric multiple comparison test for unequal sample sizes was used to test for significant differences in trace metal concentration between sites (Hollander and Woolf, 1973).

RESULTS

Metal levels in natural populations of M. edulis, C. virginica and C. gigas are found in Table 1. These data were used to determine the tissues used in the transplant experiment. Oyster gills and whole bodies showed large differences in metals between the control sites (Tomales Bay and Elkhorn Slough) and the relatively polluted site (Redwood City). We decided to use gills because:

1. These data indicated that gills concentrate metals as well as whole bodies; e.g., oysters.

2. Previous studies have shown whole body tissues to vary seasonally in trace metal concentrations. This may be due to seasonal changes in gonad development. Thus, gill tissue would not be expected to vary seasonally as much as whole bodies. This is particularly important in transplantation experiments in which collections are made when the population had both spawned and unspawned organisms.

3. There is little problem with sediment contamination of gill tissues.

TABLE 1: Trace metal levels in resident mussels and oysters
(Means in µg/gm dry weight ± standard deviation)

	Location	No. Samples	Cd	Cu	Zn	Mn	Fe	Ag
Crassotrea virginica								
gills	Tomales Bay	9	27.6± 5.7	295±131	3560±2310	28.1± 5.5	288±180	5.5± 2.9
	Redwood City	10	49.5±11.4	1810±372	19900±6450	29.7± 7.3	159± 51	145 ±46
whole bodies	Tomales Bay	10	12.2± 4.3	273±117	3570±1220	34 ±18.7	260±207	6.4± 2.8
	Redwood City	10	39.7±10.9	1510±702	19300±8970	13 ± 3.3	148± 27	81.5±56.7
Crassostrea gigas								
gills	Tomales Bay	10	20.3± 6.8	178± 58	290± 100	125± 43.8	270± 81	3.7± .99
	Redwood City	10	56.4±14.2	1910±430	8040±1590	111± 38.1	580±511	189 ±40.4
whole bodies	Tomales Bay	10	9.0± 3.9	86± 30	442± 134	40± 16.0	368±149	1.7± .50
	Redwood City	9	40.9± 9.4	860±287	3550±1440	61± 18.0	668±274	82 ±36
Mytilus edulis								
whole bodies	Elkhorn Sl.	30	11.2± 4.3	19± 6	161± 96	6.6± 2.7	433±195	.18± .05
	Redwood City	28	12.8± 4.2	30± 6.7	281± 182	74.3±42.4	402±177	.60± .59

		Pb	Al	Ni	Cr
Elkhorn Sl.	30	.36± .21	274±163	1.9± .95	1.7±.82
Redwood City	28	2.8 ±2.3	336±172	9.0±2.7	2.0±.72

M. edulis whole bodies showed large differences between Redwood City and Elkhorn Slough, although these were not always of the same magnitude as the oyster differences.

Results of the individual analyses of oyster gill tissue from Drake's Bay and the four transplantation sites are shown in Table 2; individual analyses of the mussel gill tissue from Tomales Bay and the four transplantation sites in Table 3. Species differences in gill tissue uptake are evident, with oyster gill accumulating higher concentrations of all metals.

Statistically significant ($p < .05$) differences between control and transplantation sites were found for some metals. Both oysters and mussels at Redwood City contained significantly higher levels of Cu than the control organisms. In addition, the Redwood City oysters had significantly higher Ag and Cd levels. The Monterey sewage outfall site had significantly higher levels of Ag in oysters and of Ag and Cu in mussels, compared to the control sites. Mussels at Elkhorn Slough had significantly higher Cu levels than at the control area. Levels of Fe and Ms in oysters ($p < .15$) were significantly lower at the Monterey sewage outfall site compared to the respective control site, evidence of significant depuration.

Accumulation rates in terms of concentration increase per month are shown in Table 4 A. Oysters at every transplantation site accumulated up to forty times more Ag, Cu and Cd (except for Cd at Monterey sewage outfall) than mussels. They also had a higher depuration rate for Mn and Fe than mussels. In addition, Redwood City oysters accumulated more Ag, Cu and Cd than Monterey sewage outfall oysters. Mussels, however, accumulated slightly less Ag, Cu and Cd at Redwood City than at the Monterey sewage outfall. Redwood City mussels and oysters accumulated high concentrations of Mn in comparison to the other transplantation sites. Monterey sewage outfall oysters accumulated high levels of Ag and Cu, almost no Cd and depurated more Mn and Fe than those at the other stations. Metal accumulation rates at Elkhorn Slough and Granite Canyon were generally low except for Cu in mussels at Elkhorn Slough. Zn data did not show significant trends at any station.

Accumulation rates expressed as percent increase per month are shown in Table 4 B. They show the same trends as the concentration increase per month rates in Table 4 A, with the following exceptions: Redwood City did not always have higher uptake rates than Monterey sewage outfall; for example, Monterey sewage outfall mussels had a higher percent increase rate than Redwood City mussels in Ag and Cu. Also, mussels sometimes had a higher accumulation rate than oysters. For example, mussels at Monterey sewage outfall and Elkhorn Slough had higher uptake rates of Ag and Cu than oysters.

TABLE 2: Metal concentrations in oyster (C. gigas) gills one month after transplantation

Location	Replicate Number	Ag	Cd	Cu	Fe	Mn	Zn
		µgm/gm dry weight					
Drake's Bay (control)	1	1.8	6.4	41.7	233	119	1810
	2	1.4	7.9	43.4	271	44	2350
	3	1.0	10.0	18.5	302	148	1460
	4	2.2	10.6	48.8	304	160	2470
	5	1.9	11.9	42.1	344	53	2790
	6	2.5	14.0	56.1	188	98	2980
	7	1.6	6.2	56.8	212	70	2200
	8	.9	13.1	32.7	354	86	2000
	9	1.9	10.3	29.6	302	58	2030
	10	1.9	8.7	34.1	256	77	1880
	$\bar{X}$±95% cc	1.7±.4	9.9±1.9	40.4±8.6	277±39	91±29	2200±330
	Median	1.9	10.1	41	286	81	2110
Granite Canyon	1	2.6	14.9	35.1	218	47	1530
	2	3.0	11.1	73.2	184	65	3540
	3	2.5	17.6	54.1	305	37	2480
	4	2.2	11.2	23.2	260	21	1460
	5	2.2	9.4	47.4	209	24	1880
	6	2.1	9.5	50.7	283	45	2320
	$\bar{X}$±95% cc	2.4±.4	12.3±3.4	47.3±17.9	243±49	40±17	2200±810
	Median	2.0	11.1	49.1	239	41	2097
Monterey Sewer Outfall	1	4.8	9.6	69.1	154	12	2330
	2	2.5	12.9	26.8	166	26	2260
	3	5.4	13.2	62.9	204	33	1830
	4	4.3	9.5	53.9	178	56	1950
	5	5.0	9.6	41.6	233	50	1840
	6	5.3	16.3	67.8	169	28	2250
	7	5.1	8.3	49.1	129	16	1500
	8	5.2	23.7	58.0	230	29	2790
	9	3.9	6.8	48.1	249	58	1820
	$\bar{X}$±95% cc	4.6±.7	12.2±4.0	53.0±11.4	190±31	34±13	2060±290
	Median	5.0	9.6	53.9	178	29	1950
Elkhorn Slough	1	.5	20.4	23.9	183	89	1220
	2	1.9	12.7	46.8	308	29	1960
	3	2.1	6.8	62.9	192	45	1940
	4	.9	12.7	22.6	339	42	740
	5	2.5	12.1	40.4	249	52	2100
	6	1.2	9.8	41.5	251	38	1230
	7	3.3	15.7	72.7	213	64	1800
	8	1.7	9.4	46.0	151	60	1510
	9	2.7	10.7	80.0	233	42	3060
	$\bar{X}$±95% cc	1.9±.7	12.3±3	48.5±15.3	235±46	51±14	1730±510
	Median	1.9	12.1	46.0	233	45	1955

TABLE 2: Metal concentrations in oyster (C. gigas) gills one month after transplantation

Location	Replicate Number	Ag	Cd	Cu	Fe	Mn	Zn
		µgm/gm dry weight					
Drake's Bay (control)	1	1.8	6.4	41.7	233	119	1810
	2	1.4	7.9	43.4	271	44	2350
	3	1.0	10.0	18.5	302	148	1460
	4	2.2	10.6	48.8	304	160	2470
	5	1.9	11.9	42.1	344	53	2790
	6	2.5	14.0	56.1	188	98	2980
	7	1.6	6.2	56.8	212	70	2200
	8	.9	13.1	32.7	354	86	2000
	9	1.9	10.3	29.6	302	58	2030
	10	1.9	8.7	34.1	256	77	1880
	$\bar{X}$±95% cc	1.7±.4	9.9±1.9	40.4±8.6	277±39	91±29	2200±330
	Median	1.9	10.1	41	286	81	2110
Granite Canyon	1	2.6	14.9	35.1	218	47	1530
	2	3.0	11.1	73.2	184	65	3540
	3	2.5	17.6	54.1	305	37	2480
	4	2.2	11.2	23.2	260	21	1460
	5	2.2	9.4	47.4	209	24	1880
	6	2.1	9.5	50.7	283	45	2320
	$\bar{X}$±95% cc	2.4±.4	12.3±3.4	47.3±17.9	243±49	40±17	2200±810
	Median	2.0	11.1	49.1	239	41	2097
Monterey Sewer Outfall	1	4.8	9.6	69.1	154	12	2330
	2	2.5	12.9	26.8	166	26	2260
	3	5.4	13.2	62.9	204	33	1830
	4	4.3	9.5	53.9	178	56	1950
	5	5.0	9.6	41.6	233	50	1840
	6	5.3	16.3	67.8	169	28	2250
	7	5.1	8.3	49.1	129	16	1500
	8	5.2	23.7	58.0	230	29	2790
	9	3.9	6.8	48.1	249	58	1820
	$\bar{X}$±95% cc	4.6±.7	12.2±4.0	53.0±11.4	190±31	34±13	2060±290
	Median	5.0	9.6	53.9	178	29	1950
Elkhorn Slough	1	.5	20.4	23.9	183	89	1220
	2	1.9	12.7	46.8	308	29	1960
	3	2.1	6.8	62.9	192	45	1940
	4	.9	12.7	22.6	339	42	740
	5	2.5	12.1	40.4	249	52	2100
	6	1.2	9.8	41.5	251	38	1230
	7	3.3	15.7	72.7	213	64	1800
	8	1.7	9.4	46.0	151	60	1510
	9	2.7	10.7	80.0	233	42	3060
	$\bar{X}$±95% cc	1.9±.7	12.3±3	48.5±15.3	235±46	51±14	1730±510
	Median	1.9	12.1	46.0	233	45	1955

TABLE 2 (Continued)

Location	Replicate Number	Ag	Cd	Cu	Fe	Mn	Zn
				µgm/gm dry weight			
Redwood City	1	13.3	10.8	190	220	102	3020
	2	38.1	18.2	323	244	79	4000
	3	10.5	15.8	108	210	180	1250
	4	21.8	19.6	209	309	69	2740
	5	12.0	18.0	214	252	105	4070
	6	26.6	20.9	236	239	79	3520
	7	22.9	19.9	167	156	41	2410
	8	22.2	12.7	215	227	148	2380
	$\bar{X}$±95% cc	20.9±7.6	17.0±3.0	207±51	232±36	100±37	2920±790
	Median	22.0	18.1	212	233	85	2880

TABLE 3: Metal concentrations in mussel gills one month after transplantation

Location	Replicate Number	Ag	Cd	Cu	Fe	Mn
		µgm/gm dry weight				
Tomales Bay (control)	1	.04	1.5	5.4	168	7.6
	2	.02	1.0	4.2	130	6.0
	3	.06	1.6	5.5	126	9.1
	4	.26	1.1	5.3	212	7.5
	5	.03	4.9	7.6	160	10.9
	6	.18	3.7	5.2	161	7.1
	7	.03	1.6	6.0	125	7.4
	8	.49	.8	8.1	184	6.0
	$\bar{X}$±95% CL	.14±.14	2.0±1.2	5.9±1.1	158±25	7.7±1.4
	Median	.04	1.5	5.4	160	7.4
Monterey Sewer Outfall	1	.39	1.4	10.2	90	5.2
	2	.51	2.7	9.1	88	5.0
	3	1.06	2.0	10.9	93	5.5
	4	.32	2.9	7.6	76	4.5
	5	.35	2.0	11.4	92	6.9
	$\bar{X}$±95% CL	3±.38	2.2±.8	9.8±1.9	88±9	5.4±1.1
	Median	[illegible]	2.0	10.2	90	5.2
Elkhorn Slough	1	.05	8.2	10.3	135	9.3
	2	.06	4.5	10.4	321	9.3
	3	.03	2.3	9.5	198	7.1
	4	.07	2.8	8.7	266	9.1
	5	.05	.9	9.2	174	6.8
	6	.07	1.4	10.7	184	7.5
	$\bar{X}$±95% CL	.06±.02	3.4±2.8	9.8±.8	213±28	8.2±1.2
	Median	.06	2.5	9.9	191	8.3
Redwood City	1	.29	2.1	11.0	149	46.9
	2	.46	1.9	10.0	133	16.3
	3	.26	1.5	9.5	261	30.9
	4	.13	4.6	9.5	83	15.4
	5	.39	1.2	10.7	182	20.8
	6	.12	3.9	7.4	120	21.1
	7	.21	1.4	8.8	159	21.8
	8	.28	4.2	9.4	163	10.0
	9	.44	6.2	14.1	211	24.6
	$\bar{X}$±95% CL	.29±.10	3.0±1.4	10.0±1.4	162±40	23.1±8.2
	Median	.28	2.1	9.5	163	21.0

TABLE 4 A: Uptake rates in transplanted organisms.
Concentration increase per month[1]

	OYSTERS				MUSSELS			
	Redwood City	Monterey Sewer	Elkhorn Slough	Granite Canyon	Redwood City	Monterey Sewer	Elkhorn Slough	Granite Canyon
Ag	20.1*	3.1*	0	0.1	0.24	0.3*	0.02	--
Cu	171.0*	13.0	5	8.0	4.10*	4.8*	4.50*	--
Cd	8.0*	-.6	2	1.0	0.60	0.5	1.00	--
Mn	4.0	-52.0	-36	-40.0	13.60	-2.2	0.90	--
Fe	-53.0	-108.0	-53	-47.0	3.00	-70.0	31.00	--
Zn	764.0	-163.0	-158	-16.0	--	--	--	--

TABLE 4 B: Uptake rates in transplanted organisms.
Percent increase per month[2]

	OYSTERS				MUSSELS			
	Redwood City	Monterey Sewer	Elkhorn Slough	Granite Canyon	Redwood City	Monterey Sewer	Elkhorn Slough	Granite Canyon
Ag	1089.0*	170.0*	2.7	27	600.0	875.0*	37	--
Cu	417.0*	31.0	12.0	20	75.0*	88.0*	83*	--
Cd	78.0*	-0.5	19.0	10	40.0	33.0	70	--
Mn	4.9	-64.0	-44.0	-49	184.0	-30.0	12	--
Fe	-18.5	-38.0	-19.0	-16	1.9	-32.5	19	--
Zn	36.0	-0.8	-7.0	-1	--	--	--	--

*P < .05 that the controls differed significantly from this value.

[1]Concentration increase/month = (median ppm of experimental site - median ppm at control site)/1 month.

[2]Percent increase/month = (median ppm of experimental site - median ppm of control site)/(median ppm of control site x 1 month).

Table 5 shows the percentage of the trace metal content of four-year-old resident oysters accumulated in one month by transplanted oysters at Redwood City. The data show 10.5% of the Ag, 16.5% of the Cd, 9% of the Cu, 4% of the Mn and 9.5% of the Zn were accumulated in one month. This indicates that the oysters had only accumulated a small fraction of the metals found in resident oysters. It also indicates that, if uptake were constant, it would take less than one year to accumulate a trace metal load equal to that of natives. Presumably, then, the oysters accumulate trace metals at diminishing rates which may approach zero, thus establishing an equilibrium.

DISCUSSION

Areas which are relatively high in trace metals can be identified by using significance tests on transplant and control data. This technique was used in identifying Redwood City as high in Ag, Cd and Cu, Monterey sewage outfall high in Ag and Cu and Elkhorn Slough high in Cu. One might expect Redwood City and the Monterey sewage outfall to be high in several trace metals because of industrial and municipal waste discharges, and Elkhorn Slough to be high in Cu because of the proximity of the transplants to the cooling water discharge from the copper-nickel condensing system in the fossil fuel power plant (approximately 100 m away). However, because of the difficulties encountered using conventional monitoring techniques, the elevated concentrations of these elements have previously gone undetected.

One should use accumulation rates with some caution. If accumulation rates are assumed to be proportional to the levels of trace metals in the environment, then the accumulation rates could provide a relative measure of the degree of trace metal contamination at each site. However, we have found accumulation rates at a given site to be species-dependent. The difference in uptake rates between the two species could be due to a variety of factors, among them:

1. The two species feed on different types of suspended particulates and, if uptake through ingestion of food is important, the difference in trace metals in their food may cause the difference. However, Cd uptake in C. virginica through ingestion of food is only one-tenth the amount absorbed directly from solution (Kerfoot and Jacobs, 1976).

2. These two species favor slightly different habitats. The oysters typically favor quiet waters, while M. edulis can occur near the mouths of harbors where the wave action is stronger. This may explain the higher uptake of some metals by mussels at the Monterey sewage outfall and the higher uptake of metals by oysters at Redwood City.

3. Mussels may have reached their accumulation capacity at Redwood City, Monterey sewage outfall and Elkhorn Slough (see Table 4 A). The concentrations of Ag and Cd are nearly equal at Redwood City and Monterey

TABLE 5: Percent of metal concentrations in resident four-and-one-half-year-old oysters accumulated by transplanted oysters in one month at Redwood City.

	Median Concentration of Control Transplants	Median Concentration of 1-Month Transplants	Median Concentration Accumulation in 1 Month (from Table 46)	Median Concentration of 4-Year-Old Residents	% Accumulation in One Month by Transplantation
Ag	1.9	22	20.1	192	10.5
Cd	10.1	18.1	8	48	16.5
Cu	41	212	171	1891	9.0
Fe	286	233	-53	427	--
Mn	81	85	4	101	4
Zn	2110	2880	764	8012	9.5

*Median concentration of 1-month oysters/median concentration of 4-year-old oysters x 100%.

sewage outfall. The concentrations of Cu are approximately equal for these two sites and Elkhorn Slough as well. It may be that the mussels were accumulating at all sites at a maximum rate and could not accumulate more at Redwood City if it were available. If this is the case, transplants should be left out long enough to reach equilibrium with the environment. In future studies, uptake should be determined over several time intervals. The rate could then be calculated from a curvilinear function based on several measurements. Perhaps then, species accumulation rates may be more accurately compared.

The transplantation technique should be evaluated further using several species, tissues and time intervals.

CONCLUSIONS

Significant differences in concentrations of Ag, Cd, Cu and Mn between control and transplanted oysters and mussels were observed. This indicates that the transplantation method is useful for detecting the presence of elevated concentrations of these metals. This method can be used as a valuable monitoring tool to inexpensively survey discharges to determine whether more expensive conventional monitoring of water quality parameters should be initiated or intensified. Species differences in uptake rates suggest that, in future studies, several species, tissues and time intervals should be used.

SECTION 3

GENERAL STUDIES ON METALS IN MARINE ORGANISMS

A. Premature Pupping in California Sea Lions

Scientists involved in environmental pollution research are often required to perform detective work; i.e., mortalities or deleterious effects are observed for a group of organisms and the question arises: Was an environmental pollutant responsible and, if so, which one? Answering such questions is very difficult because of the complexities that are almost always encountered.

This situation is exemplified by studies on premature pupping in the California sea lions (Zalophus californianus). Large numbers of premature pups have been counted on the sea lion rookeries since 1968 (see Gilmartin, et al, 1976; Odell, 1970). Research aimed at determining the causes for these events have revealed that: (1) The mothers of premature pups are usually only six to eight years old, while the mothers of full-term pups are at least ten years old (Gilmartin, et al, 1976). (2) Many of the abnormal mothers are infected with Leptospirosis, a virus known to cause abortions (Vedros, et al, 1971; Gilmartin, et al, 1976). (3) The abnormal mothers have significantly higher amounts of polychlorinated biphenyls (PCBs) and DDT compounds (DeLong, et al, 1973; Gilmartin, et al, 1976). (4) The normal mothers have significantly higher amounts of mercury, selenium and bromine (Martin, et al, 1976). The latter findings were of interest because each normal mother had equimolar amounts of Se and Hg in their livers. In addition, excess or equimolar amounts of Br were always found in conjunction with these elements. In contrast, the mothers of premature pups had equimolar amounts of Se and Hg; however, Br levels were severely depressed. Perhaps these findings indicate that Br is also involved in the Hg-Se detoxification mechanisms (see Parizek, et al, 1971) and, for some reason, it was not functioning in the abnormal mothers. Whether it was responsible for the premature pupping is unknown. However, these results suggest that absolute amounts of elements are not as important as the relationship between elements.

In addition to demonstrating the complexities involved with environmental detective work, the four factors mentioned above also point to the desirability of simultaneous measurement of different pollutant classes, as well as natural factors within the same samples. Erroneous conclusions can be reached when only one pollutant is measured. As the Se-Hg interaction indicates, this is espe-

cially true for one heavy metal.

Research of this nature is extremely important in spite of the complexities involved in attempts to interpret findings. For example, the cause of eggshell thinning in the brown pelican was suggested when scientists found high DDT levels in association with these birds. Subsequent research led to the ban of the use of this pesticide in the United States. On the other hand, the detective work may yield no firm conclusions. This is the present case with the sea lions. Nevertheless, reproductive failure (premature pupping) has also been reported for harbor seals in San Francisco Bay and Puget Sound. Perhaps studies on these animals will shed more light on the causes for these events. Whenever deleterious effects are observed, multifaceted research should be performed to determine whether anthropogenic and/or natural causes were responsible.

An abstract of our sea lion work follows.

Martin, J.H., P.D. Elliott, V.C. Anderlini, D. Girvin, S.A. Jacobs, R.W. Risebrough, R.L. Delong and W.G. Gilmartin. 1976. Mercury-selenium-bromine imbalance in premature parturient California sea lions. Mar. Biol. 35:91-104.

Abstract: High premature birth rates have been observed in the rookeries of the California sea lion Zalophus californianus since 1968. The reasons for the premature pupping are complex and, hence, not well understood, although leptospirosis infection and elevated PCB and DDT residues have been implicated. We were interested in determining what role trace and major elements played in these events. Livers and kidneys from ten normal parturient and ten premature parturient mothers and their pups were analyzed for Hg, Se, Br, Cd, Ag, Cu, Fe, Zn, Mn, K, Na, Ca and Mg in order to detect differences that might exist between the two groups. A further objective was to establish how these elements varied in relation to each other in the normal and abnormal sea lions. Our results revealed that Hg, Se, Cd and Br levels were significantly higher in the livers of the normal mothers and that these elements were all in balance (highly correlated) with each other. This was especially true for Hg, Se and Br. In mothers with high concentrations of these elements (e.g., Hg greater than 800 µg/g dry weight), atomic ratios of approximately 1Hg:1Se:1Br were observed. Atomic Se:Hg ratios were also near unity in the abnormal mothers; however, Br concentrations were always severely depressed in these individuals. Normal full-term pups had higher hepatic levels of Hg and Se, and near-perfect 1:1 Se:Hg atomic ratios were almost always observed. In contrast, the livers of the premature pups appeared to be deficient in Hg, and, consequently, elevated Se:Hg ratios were always found. In almost all cases, the premature pups had increased concentrations of Na, Ca and Br. Levels of these elements were correlated with their Se:Hg ratios. Amounts of Mn and Cu were reduced in the premature pups and negatively correlated with Se:Hg ratios. The results suggest that balance between elements is of more importance than absolute concentration when the possible effects of toxic elements are considered. It also appears that bromine may be important in the detoxification process involving Se and Hg and perhaps Cd as well; i.e., every mother that had Br in balance with Hg, Cd and Se had a

normal pup, while every mother that lacked sufficient Br had a premature pup. The question of whether Hg detoxifies Se is also raised. All the normal pups had Se:Hg atomic ratios of less than 2.2, while all the premature pups had reduced Hg amounts and Se:Hg ratios above 3.4.

B. Silver, Cadmium, Copper, Zinc and Iron in Squid Livers

We were especially interested in squids, not only because of their enormous importance in the pelagic food web (Clarke, 1966, p. 265), but also because they concentrate silver (Folsom and Young, 1965; Folsom et al, 1970), an element well known for its toxicity to marine organisms (Soyer, 1963; Bryan, 1971; Calabrese, et al, 1973). The measurement of this metal in liver tissue, where it is known to concentrate, was thus the primary objective of our research. In addition, we wanted to compare silver concentrations with levels of three other closely related elements: copper, cadmium and zinc. Iron was also determined for background information.

The most significant finding in the study was the enormous copper concentrations in the Loligo opalescens livers and the correlations that exist between this element and silver and cadmium. The two most plausible explanations would seem to be: (1) Since squids require copper for the synthesis of their respiratory pigment, hemocyanin, these high levels may be entirely natural; in the process of concentrating Cu, some incidental uptake of Ag and Cd occurs. (2) Silver and/or cadmium may cause copper to be concentrated in the liver of L. opalescens; this process thus reflects a sub-lethal effect caused by these heavy metals. Both metals are well known for their toxicity and both are known to affect copper metabolism, at least in birds and mammals (e.g., Anke, et al, 1970). In laboratory studies with rats, Van Campen (1966, p. 129) noted that the "major effects of silver were a decrease in the relative proportion of ^{64}Cu in blood and an increase in the proportion deposited in the liver". In general however, these processes are only poorly understood. Underwood (1971, p. 76) states: "The diversity of the physiological responses and the interactions with copper produced by the four elements, zinc, cadmium, silver and mercury, which are chemically very similar, is surprising and, at present, inexplicable".

These relationships have also been observed in other molluscs. Windom and Smith (1972) reported correlations between Cu:Ag, Cu:Zn and Ag:Zn in East Coast (USA) oysters. Anderlini (1974) also noted a negative correlation between silver and copper in West Coast abalone. Thus, representatives of three classes of mollusc are known to have these relationships.

The abstract of our squid livers paper follows.

Martin, J.H. and A.R. Flegal. 1975. High copper concentrations in squid livers in association with elevated levels of silver, cadmium and zinc. Mar. Biol. 30:51-55.

Abstract: Livers from 43 Loligo opalescens, 14 Ommastrephes bartrami and 7 Symplectoteuthis oualaniensis were analyzed for their silver, cadmium, copper, zinc and iron contents. Copper concentrations of up to 15,000 µg/g

dry weight were found in L. opalescens in conjunction with significant correlations between this element and Ag, Cd and Zn. The latter elements are known to affect Cu metabolism in terrestrial organisms; however, whether the correlations occurring in marine organisms represent casual or cause-and-effect relationships is as yet unknown.

C. Abalone Copper Toxicity Experiments.

In cooperation with the California Department of Fish and Game, we took part in copper toxicity studies that demonstrated that adult abalone died when subjected to levels of 65 and 50 ug Cd/liter. These findings supported the contention that 1,500 abalone died from copper poisoning when the cooling system of the power plant was tested.

Martin, M., M.D. Stephenson and J.H. Martin. 1977. Copper toxicity experiments in relation to abalone deaths observed in a power plant's cooling waters. Calif. Fish and Game, 63(2):95-100.
Abstract: Toxicity of copper as copper sulfate and larval red abalone, Haliotis rufescens, and adult black abalone, Haliotis cracherodii, was determined by static bioassay in seawater at 14 °C (57 °F). Copper accumulation studies and histopathological analysis in digestive gland and gill tissues were conducted. The TL_{50}s for adult red and black abalone were 65 ppb and 50 ppb copper, respectively. The TL_{50} for larval red abalone was 114 ppb copper. Copper was found to accumulate in gill tissues of red and black abalone at 56 ppb copper concentration. Histopathological abnormalities in gill tissues occur at concentrations above 32 ppb.

D. Cadmium in Plankton: Elevated Levels off Baja, California.

On three occasions, we observed elevated cadmium levels in plankton collected off Baja, California. These findings were described in an article published in Science:

Martin, J.H. and W.W. Broenkow. 1975. Cadmium in plankton: elevated concentrations off Baja California. Science 190:884-885.
Abstract: One hundred thirty-five plankton samples were collected in the northeast Pacific Ocean and analyzed for their cadmium content. Concentrations were generally low (2 to 5 micrograms of cadmium per gram, dry weight) in all samples except for the plankton collected off Baja California, where high values (10 to 20 parts per million) were consistently found on two cruises.

A question familiar to all researchers studying heavy metals in the marine environment was raised: Is the element in question derived from natural or anthropogenic sources? In this case, we postulated that the Cd could either be concentrated naturally in these waters by upwelling processes (Schutz and Ture-

kian, 1965; Riley and Taylor, 1972) or it was from some unknown anthropogenic source. Obviously, the only way to find out was to measure cadmium in sea water.

Under the auspices of the NSF IDOE program, we began working on this problem in January of 1975. After perfecting methodology (see Martin, et al, 1976b), we were able to successfully collect sea water at several depths without contaminating the water with extraneous amounts of Cd.

In our Science article, we postulated that the reason for the high levels was that Cd concentrations in the water must also be elevated here. However, when we measured the amounts in the water, we were surprised to find that surface values were extremely low (4.5 ng Cd/liter, Bruland, et al, 1978). We can now tentatively conclude that the Cd we find in the plankton in these waters is from a natural source; that is, as deep waters upwell to the surface, they will bring up plant nutrients that stimulate phytoplankton growth and, at the same time, elevated amounts of Cd will be introduced to the surface. Whether the phytoplankton also take up amounts of Cd proportional to phosphate and nitrate is unknown. However, our preliminary data suggests that the Cd is not concentrated actively. Phytoplankton collected in Monterey Bay after intense upwelling pulses contained very small amounts of Cd (1 to 2 ppm; see Martin and Knauer, 1973). Although phosphate was not measured in these samples, they should be rich in this element, since actively growing phytoplankton usually have high concentrations of P and N (Parsons and Takahashi, 1973). These results suggest that Cd uptake would occur after bloom conditions cease; that is, after a population becomes senescent. When this slow-down occurs, sufficient time would be allowed for Cd uptake to occur via passive adsorption. With sufficient time, Cd levels would increase markedly in the plankton and, at the same time, decrease in the water column. This, then, could explain the levels we found off Baja in the plankton and water. Most of the Cd had been adsorbed onto the plankton and associated levels were high (15-20 ppm). Because of this removal, dissolved amounts become low due to this extraction process (5 ppt). When this equilibrium is reached, Cd and P in the plankton also become correlated. However, if organic matter decreases, amounts of Cd would also decrease. This is suggested by two samples collected far offshore that had very little particulate P and Cd. The surface depletion and deep enrichment of Cd also suggests that Cd is removed from the surface in association with particles. As the particles sink and decompose, P and N are also released which results in the dramatic increases in the levels of these elements in the deep ocean. In concert with this process, Cd is also released.

With the discovery that Cd is correlated with plant nutrients, we are now in a position that enables us to detect superfluous amounts of this element in the water column. That is, when Cd levels lie above the predicted amounts from the nutrients present, (ng Cd/liter = -3.6 + 34.9 [umol PO_4/liter]) this can indicate anthropogenic input.

Thus, these findings suggest three things: (1) Cd levels in plankton off Baja California are apparently due to natural processes. (2) Unlike other ele-

ments, Cd is rapidly regenerated in the water column and, consequently, only limited amounts will be removed from the water column to the sediments. Thus, in areas with limited mixing, any introduction of Cd may result in increased levels within a relatively short period of time. (3) With our knowledge on the distribution of Cd in the water column and its relationship with PO_4, we are now in a position to determine anthropogenic enhancement.

E. Cadmium in Sea Otters

Measurements of heavy metals in top predators are needed for an understanding of food-chain amplification processes in the marine environment. Marine mammals have proved especially useful for mercury and several studies have been conducted in which levels were estimated in seals; e.g., Freeman and Horn, 1973; Koeman, et al, 1975; Gaskin, et al, 1972; Martin, et al, 1976a, and whales; e.g., Hall, et al, 1971. Since most of these marine mammals feed mainly on members of the pelagic food chain, it is desirable to compare heavy metals in species feeding on benthic food items. The California sea otter (Enhydra lutris) is especially interesting, as it feeds primarily on mollusks (Vandevere, 1969), which are known to readily concentrate cadmium (e.g., Anderlini, 1973; Brooks and Rumsby, 1965), an element well known for its toxicity (e.g., Nilsson, 1970). The main objective of this study was to determine the cadmium levels now existent in the California sea otter herd. Since we were interested in relating levels of this element to other heavy metals and essential elements, Ag, Hg, Cu, Zn, Fe, Mn, K, Na, Mg, Ca and Sr concentrations were determined as well. The digestion methods we used are described in Section 4.

Results

As expected, sea otters concentrate large quantities of cadmium, especially in their kidneys (Tables 4 and 5). A typical otter begins life with about 1 µg/g dry weight in its kidneys. Levels steadily increase as the animal grows up and eventually amounts as high as 964 ppm can occur in the females; up to 350 ppm in the males (Figure 3). It is also evident that female otters concentrate more Cd than males, since seven females had amounts exceeding that of the highest male (Figure 3).

The relationship between Cd and Zn is well known, and an interesting pattern was observed in the otters (Figure 4). A typical otter has approximately 100 ppm zinc in its kidneys at birth; or, in other words, 100 times as much Zn as Cd. As Cd concentrations increase, zinc levels also go up, but the rate of increase is slower than that for Cd. This results in a steadily decreasing Zn:Cd ratio, until amounts of the two elements become nearly equal (approximately 300 ppm). Eleven otters had renal Cd levels above 300 ppm; only one of these animals had over 300 ppm Zn (the female with 964 ppm Cd). Thus, it appears that Zn uptake responds to increasing renal Cd concentration, but this response appears to cease after Cd levels exceed those for Zn. This may have important

TABLE 4: Metal concentrations in female sea otter kidneys

Animal No.	Length mm	Weight Kg	µg/g Dry Weight										
			Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
1	461	1.2	0.7	75	0.09	21	503	1.96	5960	4890	437	596	0.93
2	675	2.8	1.2	92	0.72	38	316	3.40	6990	7990	649	285	1.20
3	655	3.3	1.4	86	0.28	15	453	3.42	9185	9880	800	182	1.28
4	734	3.4	2.0	110	0.54	38	587	2.20	6600	16320	917	2750	22.36
5	620	2.3	2.3	108	0.17	29	281	1.75	8770	10380	852	536	nd*
6	807	6.0	6.9	87	1.33	25	225	3.38	4100	12700	1469	627	17.61
7	806	4.8	15.5	177	0.39	45	321	4.81	8920	10900	1043	670	4.20
8	810	4.5	17.7	128	1.47	28	459	5.10	5270	8670	646	247	1.36
9	740	3.9	25.0	138	1.05	23	383	5.94	9170	9470	655	353	1.46
10	863	10.3	26.3	109	2.27	42	341	6.29	9450	5610	1122	1010	11.26
11	946	10.7	26.5	105	1.53	28	385	3.59	6870	6510	1196	620	5.18
12	1017	12.0	28.2	107	6.00	29	438	4.69	6060	13930	2476	2149	29.44
13	--**	4.4	31.2	133	0.60	120	622	7.40	8380	16855	731	283	5.46
14	1160	14.1	32.8	107	14.61	14	787	3.05	7240	6980	505	249	0.78
15	879	9.1	34.8	113	0.66	25	246	2.39	6410	5430	569	369	3.88
16	928	6.9	36.9	134	3.17	37	816	4.80	8300	6620	787	101	0.77
17	995	10.0	49.3	165	2.92	40	493	5.06	6790	15180	1198	493	4.53
18	1098	14.2	51.6	283	2.32	52	663	3.95	6840	10540	1087	667	5.96
19	974	9.2	53.1	146	8.68	29	901	3.93	8100	6910	650	151	1.83
20	836	6.4	58.9	370	2.84	47	257	3.19	5975	17200	812	717	5.42
21	985	9.1	60.4	145	5.65	54	495	5.31	6460	9780	887	217	2.17
22	1040	10.0	64.7	147	3.60	48	713	3.42	4865	10520	625	309	1.32
23	929	8.0	73.8	145	5.42	45	568	1.70	6740	9155	412	426	2.27
24	1075	14.0	78.1	172	4.21	34	552	3.13	6510	10550	430	618	5.99
25	1058	10.1	79.3	206	1.59	40	561	4.77	6970	9930	852	486	3.44
26	1075	11.2	103.4	143	7.59	45	422	6.02	6880	6520	538	452	4.59
27	910	6.5	104.1	182	5.39	45	411	4.16	6700	13005	1560	312	4.42
28	1040	10.1	106.4	194	5.95	30	461	3.49	5970	13540	840	263	1.92
29	1035	8.9	133.7	232	7.64	28	718	3.99	8380	10580	718	429	7.98
30	953	9.8	138.1	210	5.97	35	388	5.18	7770	11150	986	281	2.30

*Not detected
**Animal was decapitated

continued

TABLE 4 (continued)

Animal No.	Length mm	Weight Kg	µg/g Dry Weight Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
31	1080	11.4	198.2	292	6.24	46	549	5.92	6750	9710	1289	708	5.92
32	1225	15.6	222.9	177	3.10	30	616	3.49	8020	9820	575	343	0.93
33	1177	14.1	268.8	288	29.58	35	576	4.72	8960	9905	719	171	2.12
34	1120	11.3	284.5	305	3.83	45	401	4.66	8340	10860	724	252	1.55
35	1327	25.4	332.7	188	1.49	27	519	3.19	6520	5040	496	252	1.30
36	1216	23.0	373.6	243	2.50	15	283	3.18	6540	7430	588	371	3.32
37	1140	10.4	407.4	283	2.71	59	650	4.88	7390	15750	567	307	1.39
38	1132	11.9	418.9	268	3.69	49	470	3.87	6180	5920	396	348	1.83
39	1178	12.0	426.6	200	6.81	51	560	5.18	6720	7150	444	308	1.73
40	1250	16.1	593.9	253	2.66	31	464	3.97	8015	11080	702	424	2.26
41	1192	15.2	679.8	241	2.56	34	571	3.57	6405	8460	550	402	1.56
42	1280	16.8	964.1	382	4.20	52	632	3.99	9390	8975	550	249	2.99

TABLE 5: Metal concentrations in male sea otter kidneys

Animal No.	Length mm	Weight Kg	µg/g Dry Weight										
			Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
1	455	1.2	nd*	123	0.59	41	623	2.46	8500	16385	1405	1075	1.64
2	590	2.1	0.3	102	0.09	20	525	2.47	6335	10200	865	371	1.24
3	422	1.0	0.6	72	0.12	13	786	0.55	1800	18700	454	1108	7.76
4	584	2.1	0.7	122	0.28	47	468	3.62	7820	10370	864	346	1.98
5	520	1.8	0.8	137	0.25	66	465	4.06	8115	8540	727	376	1.65
6	490	1.5	0.8	91	0.25	28	447	2.70	9820	12275	1411	295	3.19
7	759	3.7	1.0	78	0.24	12	648	3.04	8120	5000	500	276	1.44
8	489	1.3	2.0	72	0.14	16	371	2.42	5060	4580	506	361	1.03
9	778	5.0	5.5	106	4.30	31	353	2.93	5940	34830	1426	2560	7.68
10	694	3.3	16.8	118	1.83	27	240	3.34	5550	7825	555	152	1.22
11	1010	16.9	17.3	116	2.51	66	622	4.49	5520	6170	1720	3638	32.97
12	973	8.8	29.2	99	3.00	25	589	3.83	6345	5640	539	237	0.60
13	965	9.8	32.5	137	1.44	30	353	4.25	7370	11550	927	266	4.56
14	1132	18.9	32.6	138	2.98	22	688	3.18	5500	10180	566	326	nd
15	920	8.5	34.2	155	1.10	51	487	4.55	7940	11160	882	560	6.93
16	1091	14.8	42.3	126	3.23	29	284	3.76	9450	9130	1857	9540	90.76
17	1155	20.2	44.4	116	2.12	25	308	4.19	6720	12330	1368	456	6.90
18	1069	21.1	62.8	105	25.45	57	969	4.73	6340	9290	2650	2817	20.04
19	1130	17.1	64.5	116	5.31	45	291	6.16	10105	7370	1587	910	9.28
20	1113	17.2	65.7	177	4.19	113	273	8.46	8910	6860	1894	1032	11.00
21	1031	9.8	66.0	155	2.90	25	486	4.24	7890	14050	2360	807	5.84
22	1220	20.4	68.8	126	23.59	38	541	5.83	11080	7120	653	263	3.27
23	1002	9.9	87.3	174	7.87	42	495	5.11	7250	12700	1387	905	6.96
24	1297	29.9	92.3	134	7.69	14	538	4.05	3740	25110	2350	1411	15.87
25	1263	22.9	107.1	107	3.72	26	748	2.88	5300	5440	464	263	0.94
26	1259	26.0	116.0	168	7.05	18	1083	3.67	8030	7300	1160	1209	15.09
27	1294	30.4	119.8	170	10.81	32	753	4.40	7400	6340	1414	2356	17.70
28	1225	23.9	120.8	189	7.62	23	540	4.02	8885	5760	826	357	3.17
29	945	11.1	120.9	148	5.64	85	260	5.40	8100	6880	643	386	3.86

*not detected

continued

TABLE 5 (continued)

Animal No.	Length mm	Weight Kg	µg/g Dry Weight Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
30	1225	26.1	126.8	180	5.54	43	945	5.37	9310	12540	2655	1178	9.45
31	1269	26.9	129.9	144	7.12	27	721	6.50	7605	8320	931	578	2.69
32	1323	21.3	133.4	202	13.31	20	845	6.59	9700	8870	682	599	2.51
33	1228	28.3	133.7	138	6.17	22	565	4.82	4925	7220	854	405	4.01
34	1090	15.5	135.4	179	5.16	45	602	4.70	4960	8240	959	1204	8.90
35	1075	11.0	142.2	193	2.46	40	525	3.94	7875	9950	798	448	3.72
36	1220	21.3	147.1	170	5.28	56	327	3.97	8690	10630	960	298	3.60
37	1232	23.1	155.2	191	12.44	19	599	3.30	6720	6080	599	295	1.28
38	1340	18.4	181.0	305	9.47	37	741	3.93	8260	7650	741	2782	9.47
39	1175	15.0	181.7	222	4.11	22	426	4.65	8530	6480	643	183	0.66
40	1358	29.0	195.5	221	6.21	26	924	5.55	8090	6820	1023	1012	5.00
41	1268	23.4	226.0	246	11.31	41	364	7.86	9170	7475	1058	471	2.09
42	1308	21.3	238.1	228	4.11	26	883	3.90	9235	6360	539	862	1.85
43	1170	16.6	252.3	303	22.03	48	605	5.47	8410	8725	846	173	1.47
44	1408	22.8	272.2	258	8.03	25	957	2.25	6240	9110	675	870	3.07
45	1231	20.9	302.1	296	9.12	21	572	5.94	8115	8630	585	265	4.83
46	1260	29.9	317.7	188	2.97	24	370	4.20	7860	9035	566	1718	11.60
47	1388	31.5	350.4	251	1.76	31	2110	4.27	5500	15715	702	337	4.04

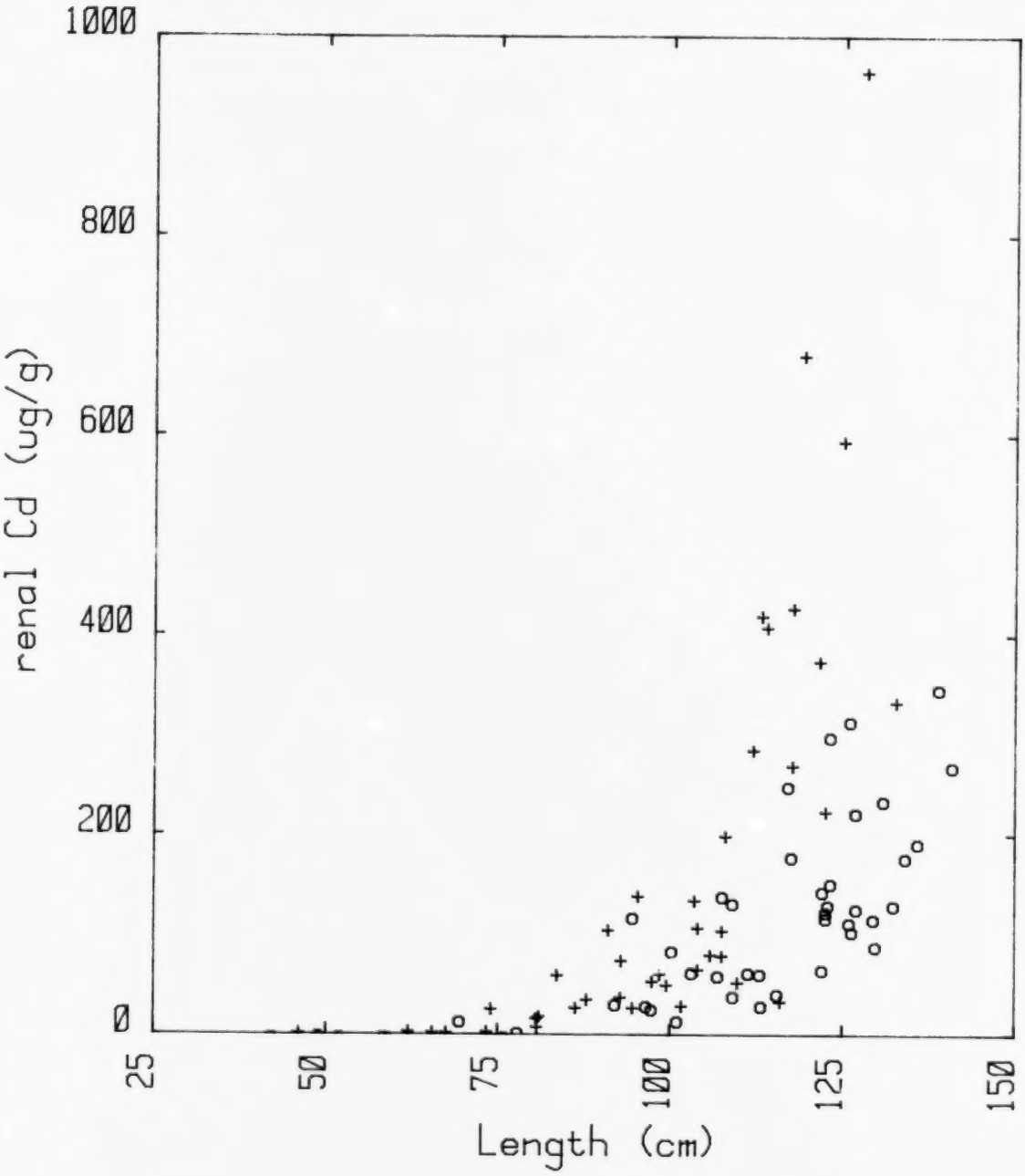

FIGURE 3: Length versus cadmium concentrations (µg/g dry weight) in sea otters. + = female; o = male.

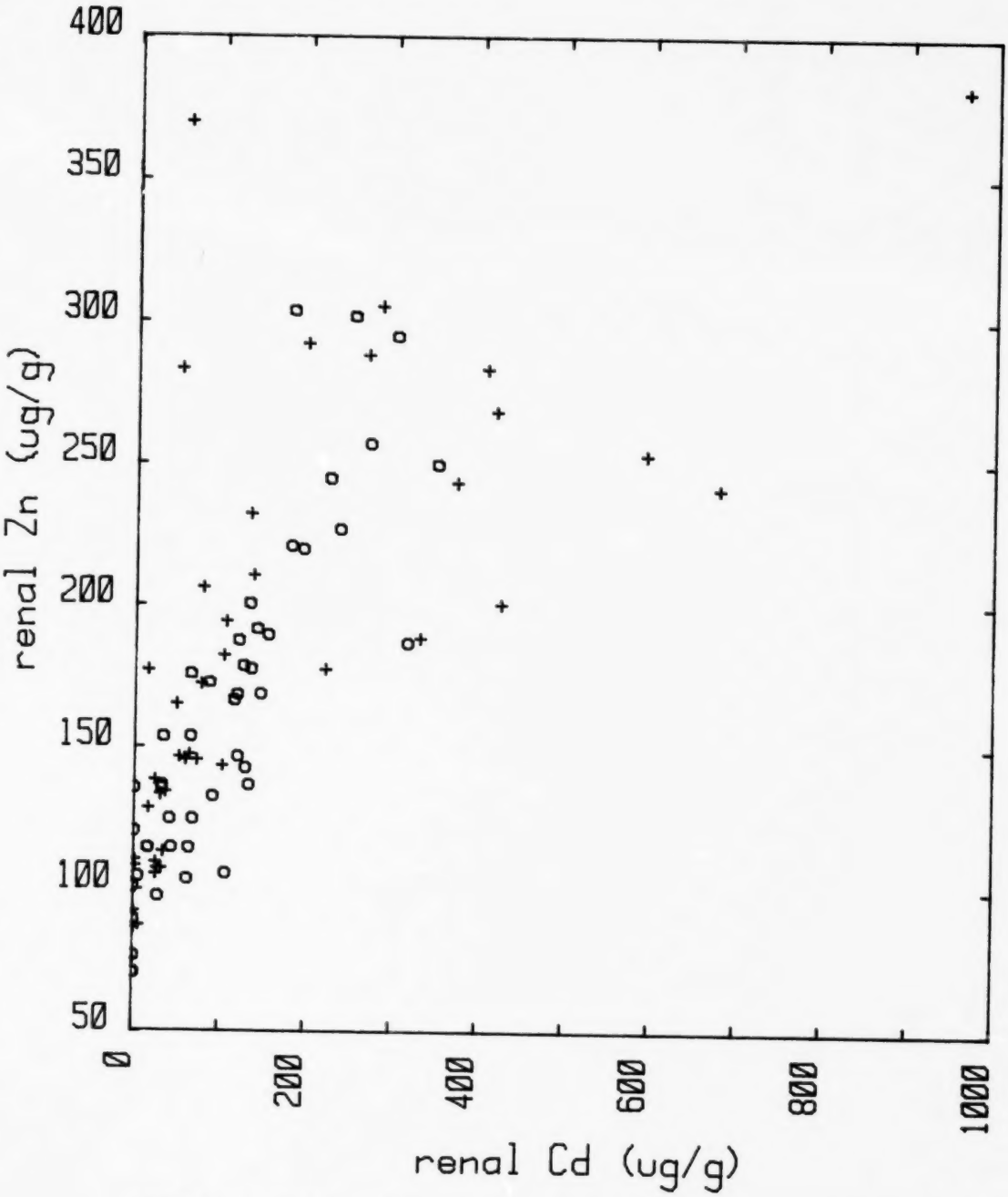

FIGURE 4: Renal cadmium concentrations versus renal zinc concentrations (both expressed in µg/g dry weight) in sea otters. + = female; o = male.

health implications, since Schroeder has reported the occurrence of hypertension in rats when a renal Cd:Zn weight ratio of .58 is exceeded (Schroeder and Buckman, 1967).

Cadmium was also concentrated in the livers of the otters (Tables 6 and 7), but levels were almost always lower than those observed in the kidneys. No relationship was apparent between hepatic Cd and Zn, however. Muscle and heart tissue samples were also analyzed for this element (Tables 8 and 9). In general, low values were found except in the animals that had exceptionally high renal and hepatic Cd concentrations. For example, Female #42, which had 964 and 665 ppm Cd in her kidneys and liver, had 19.1 and 17.0 ppm Cd in her muscle and heart tissues. This suggests that the normal detoxification organs were overloaded and Cd was allowed to concentrate throughout the animal.

In comparison to Cd, levels of mercury were relatively low in most otters; the highest amount (61.2 µg Hg/g dry weight) was found in the liver of Male #22. Hepatic Hg concentrations versus lengths are plotted in Figure 5. As was the case with Cd, mercury levels were also low in the younger, shorter otters. However, unlike Cd, adult males appear to concentrate more Hg than the females. For example, ten out of eighteen males whose length exceeded 1,200 mm, had over 10 ppm Hg, while all five females over 1,200 mm had less than 5 ppm Hg. In general, otters of intermediate length (sub-adults) appeared to concentrate Hg, regardless of sex (Figure 5). As expected, animals with high hepatic levels also had increased concentrations in their kidneys (Tables 4 and 5), muscles (Table 8) and hearts (Table 9). This is exemplified by Female #33, which had 50.6 ppm in her liver, 29.6 ppm in her kidney, 10.6 ppm in her muscle and 7.37 ppm in her heart.

Silver was found in the livers, but not the kidneys, of almost all otters and was more highly concentrated in the females than in the males (Tables 6 and 7). In general, younger otters had the highest concentrations. For example, Females #20 (41.1 ppm Ag), #28 (22.58 ppm) and #30 (20.70 ppm) were all sub-adults; the highest male, #13 (13.21 ppm), was immature.

Silver is also highly concentrated in very young otters; Males #1-8 and Females #1-5 all had relatively low Hg and Cd levels. These data suggest that the animals had not started feeding and were either newly-born or still sucklings. The high Ag concentrations (up to 9.09 ppm) suggest that this element readily passes across the placental barrier (or with the milk).

Why the females concentrate more Cd and Ag and less Hg than the male otters is unknown. Perhaps it is the result of the basic physiological differences between the two sexes. For example, all of the victims of the Ouchi-Ouchi disease in Japan were females over forty years old who had had multiple births (Shigematsu and Hasegawa, 1971). The difference may also be related to behavior; the females and a few dominant males usually occupy the larger best feeding areas, while the majority of the males are crowded into smaller, less desirable areas. Thus, feeding pressure is greater and the males may have to select food items containing less Cd and Ag and more Hg.

TABLE 6: Metal concentrations in female sea otter livers

Animal No.	Length mm	Weight kg	µg/g Dry Weight Ag	Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
1	461	1.2	0.87	0.2	181	0.35	102	852	2.56	4110	4760	450	547	1.23
2	675	2.8	3.72	1.5	243	1.10	242	1380	9.70	8265	11020	840	640	1.23
3	655	3.3	9.09	1.3	151	0.44	70	1424	8.42	7075	6990	753	220	ND*
4	734	3.4	6.63	1.2	169	1.41	118	4237	9.75	4970	17430	2130	3185	10.84
5	620	2.3	2.33	0.6	99	0.15	107	485	2.58	4220	6810	463	331	ND
6	807	6.0	6.78	14.8	205	5.37	341	1251	10.26	3770	18175	2735	2294	7.68
7	806	4.8	2.09	16.6	287	0.98	197	4413	22.38	8530	14015	1921	4538	9.21
8	810	4.5	11.47	8.9	211	4.22	223	596	21.97	3740	6655	701	133	ND
9	740	3.9	5.48	19.8	203	1.69	263	946	22.50	7420	7420	676	187	2.05
10	863	10.3	1.71	15.7	117	5.03	130	488	9.65	7350	6340	1230	3275	12.40
11	946	10.7	8.60	7.5	116	2.66	73	400	7.93	4640	4920	697	418	4.85
12	1017	12.0	1.93	15.9	161	17.77	177	855	9.87	4440	7590	1283	1135	11.58
13	--**	4.4	8.26	16.0	168	0.60	312	546	20.93	5820	18270	950	309	4.73
14	1160	14.1	0.13	6.3	121	25.40	54	3174	10.50	5950	5200	617	169	1.47
15	879	9.1	0.85	31.3	179	0.87	101	301	11.33	5470	4450	550	92	1.05
16	928	6.9	5.31	16.2	168	5.27	272	1120	23.69	5180	4660	673	113	0.39
17	995	10.0	3.90	21.0	212	4.82	56	678	21.47	4360	17450	2310	2056	8.96
18	1098	14.2	9.22	111.5	190	3.50	126	1417	17.95	3030	7520	1645	632	3.78
19	974	9.2	4.75	24.4	190	29.95	276	2048	22.78	7205	4600	713	98	0.94
20	836	6.4	41.10	29.9	250	4.46	140	275	29.04	5665	16040	1180	1187	4.20
21	985	9.1	2.13	21.5	155	9.52	67	751	26.56	4760	9710	1118	234	2.60
22	1040	10.0	7.91	28.9	263	7.91	126	1546	16.31	3670	9740	831	1054	2.45
23	929	8.0	1.93	62.9	262	15.26	172	443	11.82	5000	12410	882	832	3.68
24	1075	14.0	7.41	49.3	290	13.77	162	920	18.89	4460	10340	670	886	1.74
25	1058	10.1	1.47	71.4	325	4.22	163	1325	34.36	5580	8660	1066	370	3.12
26	1075	11.2	3.03	38.2	216	9.91	117	446	13.39	4690	6415	735	1159	4.94
27	910	6.5	7.50	64.6	212	9.01	127	716	28.53	7135	10415	1140	604	3.54
28	1040	10.1	22.58	43.4	298	11.02	198	516	35.37	5090	8565	892	294	ND
29	1035	8.9	7.00	31.9	251	19.10	171	1623	19.03	6025	10125	1030	1102	5.33
30	953	9.8	20.70	35.0	286	9.25	131	724	23.46	3780	7360	1064	994	2.39

*Not detected.
**Animal was decapitated.

continued

TABLE 6 (Continued)

Animal No.	Length mm	Weight kg	µg/g Dry Weight										
			Ag	Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
31	1080	11.4	4.52	73.4	287	12.21	238	1287	15.52	3445	7100	2100	1362	5.23
32	1225	15.6	2.30	78.4	260	4.56	97	1416	15.56	6165	9000	700	341	ND
33	1177	14.1	2.44	122.0	313	50.64	116	1064	19.25	6580	12960	829	378	3.62
34	1120	11.3	4.56	59.7	305	7.48	117	626	24.95	5840	8825	849	299	1.06
35	1327	25.4	1.54	121.8	164	3.39	121	676	12.04	6880	3830	556	120	0.86
36	1216	23.0	0.11	85.0	156	2.46	10	364	8.33	3750	6100	833	398	3.87
37	1140	10.4	8.72	161.6	354	5.96	138	1063	35.07	6920	6120	600	161	0.96
38	1132	11.9	9.46	175.4	313	12.99	174	1264	27.59	4925	5190	689	288	0.65
39	1178	12.0	4.05	115.1	300	18.82	189	2570	35.72	7445	8160	1000	522	0.26
40	1250	16.1	1.95	239.8	218	3.94	109	1046	19.55	4700	8180	575	153	ND
41	1192	15.2	7.09	288.5	282	3.09	104	522	19.32	5880	4700	505	142	0.83
42	1280	16.8	5.50	614.8	356	4.29	50	988	11.80	5235	11430	550	475	3.94

TABLE 7: Metal concentrations in male sea otter livers

Animal No.	Length mm	Weight kg	µg/g Dry Weight											
			Ag	Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
1	455	1.2	4.19	1.0	282	1.16	171	1968	4.51	6550	14040	1170	1472	5.72
2	590	2.1	1.58	ND*	221	0.29	220	3183	13.43	4900	10415	965	531	4.95
3	422	1.0	ND	ND	186	0.34	41	1874	0.26	2160	18730	1890	2255	12.12
4	584	2.1	5.77	0.2	243	0.31	225	1405	8.99	4650	9160	930	353	3.56
5	520	1.8	7.26	0.2	258	0.48	198	1385	9.00	6340	8500	854	506	2.28
6	490	1.5	6.27	0.7	199	0.58	191	2156	5.65	8800	14880	1850	1040	1.83
7	759	3.7	1.35	ND	180	0.44	135	1935	16.23	6910	3730	482	164	1.00
8	489	1.3	1.43	0.1	213	0.33	120	1308	3.64	4690	4970	420	357	1.15
9	778	5.0	7.11	9.6	230	11.15	358	3057	14.81	3760	29290	3330	3720	8.90
10	694	3.3	3.94	16.1	244	2.25	162	821	19.31	6750	9860	829	262	0.83
11	1010	16.9	3.58	9.4	139	6.00	179	720	3.90	3865	6515	845	1839	11.00
12	973	8.8	5.17	19.0	300	5.50	240	767	17.20	6470	7260	660	199	0.24
13	965	9.8	13.21	12.4	244	1.69	58	399	15.50	6075	13610	915	1181	6.49
14	1132	18.9	5.34	8.8	179	6.19	187	943	8.92	6145	13020	546	2189	4.52
15	920	8.5	1.46	21.4	193	2.15	119	804	34.84	3960	6270	1104	335	2.15
16	1091	14.8	0.81	18.3	161	7.53	231	1073	9.56	5885	7470	1243	3605	28.19
17	1155	20.2	2.32	13.8	166	3.28	86	356	10.39	6250	7195	1020	380	2.80
18	1069	21.1	5.07	27.3	111	23.25	179	2019	7.90	3990	7800	2034	2660	9.05
19	1130	17.1	1.00	24.1	141	13.76	123	410	10.25	6565	8470	900	531	5.47
20	1113	17.2	0.75	26.3	126	14.19	164	234	10.01	4410	5860	744	1690	5.22
21	1031	9.8	ND	47.5	275	16.55	256	1190	20.94	5470	15640	1388	1291	7.19
22	1220	20.4	2.33	31.0	165	61.23	214	1568	19.16	8930	8770	1000	817	1.47
23	1002	9.9	4.54	56.6	231	15.46	138	861	14.87	5110	10190	1059	2604	9.58
24	1297	29.9	0.53	27.8	122	17.64	45	1025	6.78	3455	15440	2035	885	10.82
25	1263	22.9	0.18	33.3	95	16.02	93	757	6.07	3740	4250	383	171	0.78
26	1259	26.0	1.13	39.9	121	4.74	40	3824	14.39	5140	5760	730	314	2.26
27	1294	30.4	ND	30.2	153	28.77	120	1372	2.93	2900	4990	284	192	5.21
28	1225	23.9	ND	62.0	206	29.27	195	1051	12.24	5475	4880	644	233	1.63
29	945	11.1	2.16	66.1	175	10.26	113	142	10.49	6370	7310	845	396	2.31
30	1225	26.1	2.02	44.7	143	4.81	122	3732	9.83	5800	8225	1903	680	5.27

*Not detected.

Continued

TABLE 7 (Continued)

Animal No.	Length mm	Weight kg	µg/g Dry Weight											
			Ag	Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
31	1269	26.9	1.75	55.1	172	5.35	75	519	10.09	6170	4440	677	52	ND
32	1323	21.3	0.62	30.4	180	22.11	68	2749	12.68	7875	4770	580	176	0.90
33	1228	28.3	1.73	46.0	158	5.49	64	589	6.48	4120	8610	1557	431	7.18
34	1090	15.5	ND	27.9	163	7.6i	95	611	6.48	3570	9925	620	796	3.39
35	1075	11.0	1.81	60.6	226	4.66	120	798	20.37	4480	9690	1118	447	2.73
36	1220	21.3	2.89	59.9	221	10.15	234	237	10.54	5220	12415	1082	536	5.29
37	1232	23.1	0.90	29.5	201	25.22	134	1282	13.34	7860	5030	667	142	1.03
38	1034	18.4	4.40	74.7	226	23.00	167	3269	19.22	5930	6250	943	444	3.54
39	1175	15.0	1.79	50.7	316	8.67	283	727	15.46	6040	7130	755	211	1.45
40	1358	29.0	0.51	49.4	153	3.88	65	1294	6.76	4580	6050	644	147	3.14
41	1268	23.4	ND	83.4	237	25.32	82	754	7.85	5370	5640	758	194	2.07
42	1308	21.3	1.66	105.1	274	6.43	116	2843	14.78	7310	6000	587	115	ND
43	1170	16.6	3.33	71.7	488	23.84	194	2789	19.95	6340	7750	847	358	1.74
44	1408	22.8	0.76	35.5	225	7.08	68	5878	16.14	5410	8820	1170	654	4.11
45	1231	20.9	ND	51.2	248	22.49	58	1744	10.11	3230	7980	984	378	5.30
46	1260	29.9	2.71	109.9	205	2.89	79	477	7.29	5450	10170	822	683	6.77
47	1388	31.5	4.56	22.3	270	7.67	133	16772	12.94	4480	14790	2058	1432	9.49

TABLE 8: Metal concentrations in sea otter muscles. Part A: Females.

Animal No.	µg/g dry weight										
	Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
2	ND*	168	0.36	9.9	250	0.75	8960	9860	813	363	1.57
3	ND	197	0.26	8.5	259	0.41	9235	8830	863	234	0.43
4	ND	204	0.25	14.7	240	0.57	7050	13700	680	1490	14.01
5	ND	87	0.01	6.7	143	ND	6970	7670	627	440	ND
6	0.2	167	0.63	18.2	381	0.66	4990	32800	2320	1580	7.84
7	ND	275	0.17	14.4	320	1.13	7630	10000	725	1730	4.10
8	ND	189	1.42	31.7	418	1.26	8610	4770	980	215	ND
11	0.1	150	1.10	10.4	344	0.61	10495	5750	1050	332	2.92
13	0.9	203	0.29	28.6	236	0.94	7730	17200	4870	994	3.97
16	ND	160	1.12	10.0	557	ND	10020	4590	864	196	ND
17	1.1	193	1.52	8.2	503	1.39	7250	16400	968	474	3.53
19	ND	185	3.53	9.5	749	1.32	12820	2560	934	93	1.23
20	3.8	190	0.84	25.7	338	2.59	9400	24700	967	683	6.82
21	ND	185	2.61	13.5	564	ND	9290	7040	888	197	ND
22	2.5	154	1.96	13.3	830	0.58	8495	6680	673	175	0.40
23	0.4	164	2.47	16.4	388	0.94	7450	8130	1250	267	0.39
24	0.5	188	3.14	9.4	662	2.42	8270	12300	486	376	2.54
25	1.4	330	1.08	10.3	514	0.56	6690	7080	600	350	2.59
26	0.6	105	3.17	6.2	420	0.32	8880	5590	569	519	4.92
27	ND	189	2.65	17.2	470	ND	8100	6940	956	320	ND
28	ND	209	2.96	11.6	536	ND	8570	9320	1020	196	ND
29	ND	186	2.80	9.1	829	1.91	8550	7800	646	219	1.40
30	1.7	164	2.32	10.7	604	0.97	7600	11400	824	322	ND
32	7.2	259	1.55	9.8	1110	0.20	6845	11800	623	260	1.97
33	1.5	213	10.61	3.4	678	2.68	5830	9060	946	194	ND
34	1.0	226	2.02	7.9	631	1.00	8705	4950	843	89	ND
35	2.1	193	0.87	7.1	480	0.23	12440	2220	838	152	1.87
36	7.0	221	1.19	7.2	536	0.33	9250	6530	800	222	2.68
37	1.4	232	--	10.1	1034	0.84	13850	3770	915	102	1.60
39	5.1	223	3.78	3.9	1153	3.15	7010	6850	963	170	ND
40	8.1	179	1.82	2.8	712	0.67	5930	9120	917	141	2.65
41	12.3	180	1.78	6.1	737	0.90	13920	3460	875	155	1.83
42	19.1	234	2.23	6.9	843	0.50	5570	5280	711	153	1.07

*Not detected.

TABLE 8: Part B: Males

Animal No.	Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
	ug/g dry weight										
1	ND*	109	0.65	13.7	391	2.06	8510	14300	1230	1040	1.68
2	ND	184	0.01	9.3	202	0.54	6910	9870	784	328	1.91
3	ND	87	0.19	16.1	526	0.54	2120	16700	1550	1970	4.58
4	ND	173	0.11	13.1	723	22.20	6790	8900	692	567	1.32
5	ND	168	0.10	17.4	265	1.08	8040	9930	795	580	3.64
6	ND	209	0.15	124.7	529	2.87	9390	10500	993	768	1.50
7	0.2	247	0.03	14.4	303	1.14	11080	7950	775	328	2.68
9	ND	147	3.28	14.3	250	ND	5930	24100	873	1110	5.48
10	ND	215	0.92	14.7	285	0.46	8440	10500	730	376	1.96
11	0.2	119	0.79	12.2	429	0.85	9050	8580	1190	1290	15.50
12	ND	225	1.66	14.1	517	0.87	9590	6890	773	314	0.69
13	ND	182	0.91	13.1	410	ND	8580	7880	733	263	0.67
14	ND	111	1.02	6.4	565	1.28	8850	4750	875	135	ND
16	1.1	188	0.77	7.4	427	0.74	5830	6500	720	290	1.72
17	ND	129	0.94	5.0	368	ND	8260	4140	875	420	ND
18	3.6	116	3.29	4.5	543	0.91	5220	7420	783	315	4.19
19	0.1	163	2.07	4.3	386	0.62	7290	5180	959	288	2.26
20	2.1	98	1.67	15.4	245	0.89	8860	7000	1510	645	4.82
21	2.5	149	2.14	10.5	595	1.21	9640	14100	653	543	4.11
22	0.5	138	6.67	4.0	514	0.74	7480	3400	693	129	ND
23	1.3	205	2.01	29.8	517	1.07	9920	22500	2070	1210	8.84
24	0.4	95	2.92	4.6	448	ND	7590	9200	1500	266	3.91
25	0.4	126	1.33	6.0	397	0.07	9130	3690	852	333	1.83
26	4.8	149	2.42	5.9	816	0.54	9700	6880	1250	129	0.95
29	0.6	114	2.58	5.4	381	0.78	10600	5940	940	175	1.02
31	0.7	106	2.44	4.9	494	ND	9920	3530	904	94	ND
32	0.8	245	5.31	7.1	650	1.05	10800	5130	722	124	1.14
34	ND	167	1.47	5.8	492	ND	6600	12200	1010	219	ND
35	3.3	237	1.33	10.1	587	1.05	9160	7550	712	276	0.66
36	0.5	116	1.37	4.7	468	ND	9530	5160	1090	120	ND
38	2.4	184	3.53	9.4	1070	0.50	8280	5120	782	308	1.93
39	ND	206	0.79	9.6	693	ND	9130	6320	950	94	ND

TABLE 8: Part B (Continued)

Animal No.	Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
	ug/g dry weight										
40	7.1	200	1.36	7.5	721	1.01	6900	4350	2240	552	3.13
42	4.1	229	3.20	8.0	783	0.30	8450	5290	785	138	0.16
43	1.5	262	4.78	6.0	783	0.89	6430	6940	687	106	ND
44	4.9	211	4.15	1.3	1210	0.35	6550	8650	568	210	ND
46	2.4	91	1.57	4.3	550	ND	8930	3980	862	82	ND
47	5.0	191	3.67	4.2	973	0.76	5020	11800	683	232	ND

* ND = Not detected.

TABLE 9: Metal concentrations in sea otter hearts

Animal No.	Cd	Zn	Hg	Cu	Fe	Mn	K	Na	Mg	Ca	Sr
						Females					
						µg/g dry weight					
2	ND*	108	0.40	41.0	371	1.10	7190	9870	825	309	ND
13	4.1	110	0.18	32.3	460	2.10	6770	14600	1220	704	5.91
20	3.6	112	0.70	31.4	502	2.46	4900	17100	1300	1120	7.09
29	3.4	99	3.16	21.8	871	2.25	3870	10200	778	492	ND
30	3.1	101	1.40	14.4	878	3.72	3740	11000	527	970	4.18
32	11.4	105	1.30	12.2	873	3.51	3580	6960	617	8340	10.80
33	7.0	98	7.37	16.6	666	2.45	3930	10700	1010	645	3.72
34	3.1	100	1.59	15.5	428	3.62	5380	10000	1130	432	3.23
39	6.6	115	3.75	19.3	475	3.70	4230	11500	788	731	ND
40	16.5	109	1.19	15.2	521	1.89	4130	12800	887	744	4.32
42	17.0	129	0.03	15.3	705	1.23	2730	12900	1320	1900	8.99
						Males					
1	ND	114	--	36.3	845	ND	6270	13300	1020	839	ND
3	ND	106	0.22	20.1	716	ND	2860	24000	3050	2150	11.10
6	ND	104	0.21	25.6	611	0.71	5180	9880	1180	800	3.70
12	0.7	91	1.43	17.6	445	0.99	5070	6610	755	242	ND
22	5.4	101	8.24	34.6	814	2.89	6500	8840	552	562	3.38
26	2.0	92	1.37	12.2	795	0.77	4290	7280	809	468	3.98
29	11.0	104	2.46	28.0	339	3.23	6480	12900	1970	608	7.85
31	1.4	84	1.15	12.0	442	1.45	6880	7330	996	340	2.46
39	1.1	99	0.61	14.7	385	2.62	5460	9650	942	501	3.93
42	2.9	79	1.83	11.4	585	1.39	4840	7980	765	345	ND
43	4.9	95	3.00	15.6	509	3.45	3920	12400	1400	593	3.87
47	3.6	82	2.45	12.4	847	2.59	2660	24000	2290	963	13.01

*Not detected.

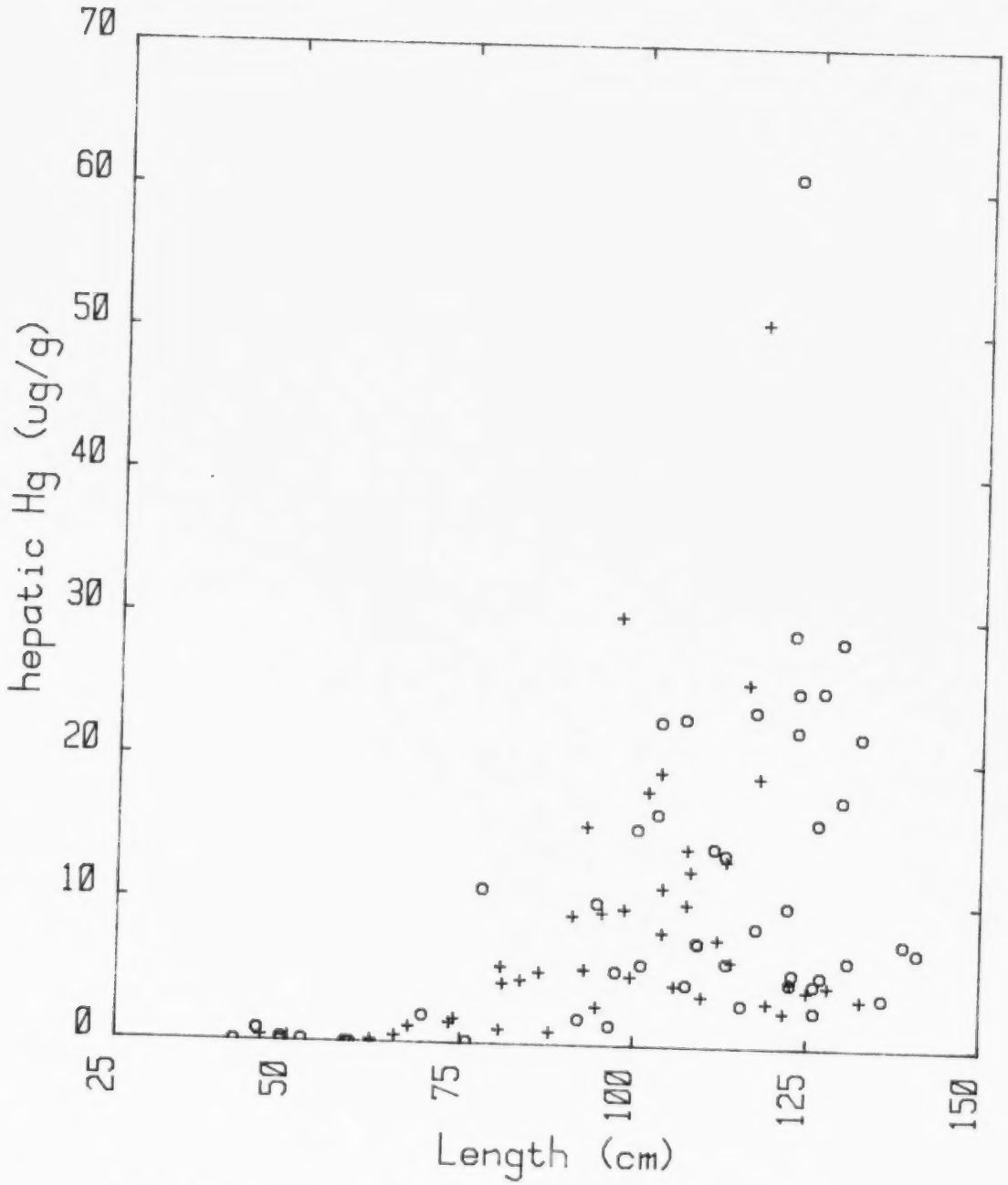

FIGURE 5: Length versus hepatic mercury concentrations (µg/g dry weight) in sea otters. + = female; o = male.

If behavior, not physiological reasons, is responsible for the observed differences in heavy metal content, we can expect changes in the various amounts of metals concentrated, regardless of sex. In recent years, feeding pressure in the growing herd has increased to the point where traditionally favored food items such as sea urchins and abalone are no longer available within the Monterey to Cambria range. This has forced many of the otters to select other prey organisms. In recent months, they have been observed feeding upon squid and, in a few cases, fish such as slow-moving cabezon (Scorpaenicthys marmoratus). If the fish become more important in the otter diet, we can expect to find increasing Hg concentrations and reduced Cd levels in future specimens. This phenomenon may already be reflected in the higher Ag and Hg levels found in more recently collected sub-adult otters of both sexes.

The remaining elements, Zn, Cu, Fe, Mn, K, Na, Mg, Ca and Sr, were measured in order to determine if concentrations of these essential elements varied in response to heavy metal levels. Many of these animals had very high Ca, Sr, Mg, Na and Fe levels in their livers (Tables 6 and 7). In almost all cases, increases in the concentrations of these elements in other organs were also observed. For example, Male #47 had 16,770 ppm Fe in its liver and 2,110 ppm in its kidney. This animal also had consistently high Na levels in its liver (14,790), kidney (15,715), muscle (11,760) and heart (24,000 µg Na/g dry weight).

Whether these abnormally high or, in some cases, low, concentrations of essential elements are the result of natural variability or a pollutant or disease is presently unknown. We will continue to work with these data in an effort to better understand their meaning. From our experience with the sea lions (Section 3-A), we have learned that the absolute concentration of a heavy metal is not as important as the relationship between that element and another element that may ameliorate its effect; i.e., Hg and Se. In the sea lions, Se:Hg imbalance appears to affect levels of several essential elements. Thus, we are curious about Se levels in the otters. Since Se has been shown to protect against Cd as well as Hg toxicity (Parizek, et al, 1971), it will be interesting to find out if the animals with high Cd levels also have high Se concentrations.

Because of the relationship we found between Mn and the ratio of Se:Hg in the aborted sea lion pups, we are especially interested in some of the otter pups that had low Mn contents. For example, Male #3 had only 0.26 ppm Mn in its liver; its K concentration was low (2160); its Ca concentration was very high (2255 ppm). Thus, chemically, this animal was very similar to the aborted sea lion pups. Department of Fish and Game personnel who collected the otter reported that it appeared to have been born prematurely. In addition to #3, there are also five males and five female pups whose livers contained less than 10 ppm Mn.

Obviously, understanding the meaning of the various data presented above is a very complicated task. Nevertheless, many interesting questions are asked that deserve answering in future studies. The otters also represent a very valuable monitoring tool. Since they are an endangered species, otter carcasses

are regularly collected and necropsies are performed. Thus, tissues are regularly made available and long-term trends in metal concentration processes can be determined. We continue to receive tissues from California Fish and Game and will soon be in a position to compare metal levels in the otter herd in the late '70s with the data we have gathered in the early '70s. These archived samples may also prove valuable in studies of other pollutants such as radionuclides and various anthropogenic hydrocarbons.

SECTION 4

METHODS

Almost all of the data presented in this report were generated using flame atomic absorption following sample digestion. Several scientists have expressed interest in our digestion procedure and, for this reason, it will be repeated in detail.

1. Weigh a 30-ml graduated pyrex beaker.
2. Place approximately 5 gm wet material in beaker and determine wet weight.
3. Place in a drying oven for 48 hours at 70-80 °C.
4. Determine dry weight (optimum weight is 1 gm).
5. Using an automatic pipet, add 5 ml redistilled 70% HNO_3.
6. Cover beaker with a 39-mm (1.5 in.) watch glass.
7. Leave samples at room temperature for 1-2 hours to allow initial frothing to subside.
8. Place beakers on a hot plate at low setting. (Watch for possible frothing; if it occurs, remove from heat and tap bottom to break large bubbles.)
9. Allow sample to reflux for approximately 30 minutes.
10. Remove watch glass and evaporate to dryness. (When sample is nearly dry, reduce heat to prevent spattering.)
11. When smoking ceases, increase heat to 340 °C. (Increase heat slowly to prevent ignition and possible loss of volatiles.)
12. After step 11, cool the sample and add 5 ml 70% HNO_3.
13. Cover beaker with a watch glass and heat at low temperature until residue redissolves.

14. With hot plate at low temperature, begin adding 30% H_2O_2, drop by drop, until solution becomes clear and a pale yellow color.

15. After clearing, evaporate solution to about 3 ml. If solution begins to darken, add additional H_2O_2.

16. Add distilled H_2O until level of solution is at the 25 ml mark on the graduated beaker. Make sure solution is well mixed.

17. With solution at room temperature, weigh the beaker and solution for viscosity corrections.

18. Analyze samples by flame atomic absorption as soon as possible.

Mercury analyses were performed using three different methods: cold vapor atomic absorption, x-ray flourescence and isotope-shift Zeeman-effect atomic absorption. These methods are discussed in the sea lion paper, Martin, et al, 1976.

Publications and Theses Based on Research Supported by EPA Grant #R 802350

Flegal, A. R. 1976. A geographic survey of silver concentrations in the gastropod Tegula funebralis (A. Adams, 1855). M. A. Thesis, California State University, Hayward.

Flegal, A. R. 1978. The geographic variation of silver in the black turban snail, Tegula funebralis. In preparation.

Flegal, A. R. 1978. Trace element concentrations of the rough limpet, Acmaea scabra, in California. Bull. Env. Contam. and Toxicol. In press.

Flegal, A. R., and J. H. Martin. 1977. Contamination of biological samples by ingested sediment. Mar. Poll. Bull. 8: 90-92.

Glickstein, N. 1977. The toxic effects of mercury and selenium in marine invertebrate larvae. M. A. Thesis, San Francisco State University.

Martin, J. H. 1976. Impact of metals on the marine environment. Paper prepared for joint American-Soviet biological seminar to be published by the U. S. Environmental Protection Agency.

Martin, J. H., and W. W. Broenkow. 1975. Cadmium in plankton: Elevated concentrations off Baja California. Science 190: 884-885.

Martin, J. H., and A. R. Flegal. 1975. HIgh copper concentrations in squid livers in association with elevated levels of silver, cadmium, and zinc. Mar. Biol. 30: 51-55.

Martin, J. H., P. D. Elliott, V. C. Anderlini, D. Girvin, S. A. Jacobs, R. W. Risebrough, R. L. DeLong, and W. G. Gilmartin. 1976. Mercury-selenium-bromine imbalance in premature parturient California sea lions. Mar. Biol. 35: 91-104.

Martin, M., M. D. Stephenson, and J. H. Martin. 1977. Copper toxicity experiments in relation to abalone deaths observed in a power plant's cooling waters. Calif. Fish and Game 63: 95-100.

Purdue, A. 1978. Mercury in sablefish, Anotlopoma fimbria, and a review of mercury in marine fish. M. A. Thesis, California State University, Hayward. In preparation.

REFERENCES

Anderlini, V. J. C. 1973. The heavy metal composition of the red abalone, Haliotis rufescens. M.S. thesis, San Francisco State University. 88 pp.

Anderlini, V. 1974. The distribution of heavy metals in the red abalone, Haliotis rufescens, on the California Coast. Arch. Environ. Contam. Toxicol. 2: 253-265.

Anderlini, V. C., J. W. Chapman, D. C. Girvin, S. J. McCormick, A. S. Newton, and R. W. Risebrough. 1975. Appendix H: Heavy metal uptake study. In U. S. Army Corps of Engineers, Dredge Disposal Study, San Francisco Bay and Estuary. U. S. Army Corps of Engineers, S. F. District, San Francisco, CA.

Anke, M., A. Hennig, H. -J. Schneider, H. Ludke, W. von Gagern and H. Schlegel. 1970. The interrelations between cadmium, zinc, copper, and iron in metabolism of hens, ruminants, and men, p. 317-320. In C. F. Mills (ed.), Trace element metabolism in animals. E. & S. Livingstone.

Bakir, F., S. F. Damluji, L. Amin-Zaki, M. Murtadha, A. Khalidi, N. Y. Al-Rawi, S. Tikriti, and H. I. Dhahir. 1973. Methylmercury poisoning in Iraq. Science 181: 230-241.

Brooks, R. R., and M. G. Rumsby. 1965. The biogeochemistry of trace element uptake by some New Zealand bivalves. Limnol. Oceanogr. 10: 521-527.

Bruland, K. W., K. Bertine, M. Koide, and E. D. Goldberg. 1974. History of metal pollution in Southern California coastal zone. Environ. Sci. Technol. 8: 425-432.

Bruland, K. W., G. A. Knauer, and J. H. Martin. 1978. Cadmium in Northeast Pacific waters. Limnol. Oceanogr. In press.

Bryan, G. W. 1971. The effects of heavy metals (other than mercury) on marine and estuarine organisms. Proc. Roy. Soc. Lond. B. 177: 389-410.

Bryan, G. W. 1973. The occurrence and seasonal variation of trace metals in the scallops Pecten maximus (L.) and Chlamys opercularis (L.). J. Mar. Biol. Ass. U. K. 53: 145-166.

Calabrese, A., R. S. Collier, D. A. Nelson, and J. R. MacInnes. 1973. The toxicity of heavy metals to embryos of the American oyster Crassostrea virginica. Mar. Biol. 18: 162-166.

Clarke, M. R. 1966. Review of the systematics and ecology of oceanic squids. Adv. Mar. Biol. 4: 91-300.

DeLong, R. L., W. G. Gilmartin, and J. G. Simpson. 1973. Premature births in California sea lions: association with high organochlorine pollutant residue levels. Science 181: 1168-1169.

Elliott, J. M. 1971. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biological Assn. Scientific Publ. No. No. 25.

Eyl, T. B. 1971. Organic-mercury food poisoning. New England J. Med. 284: 706-709.

Flegal, A. R. 1978a. The geographic variation of silver in the black turban snail, Tegula funebralis. In preparation.

Flegal, A. R. 1978b. Trace element concentrations of the rough limpet, Acmaea scabra, in California. Bull. Env. Cont. and Toxicol. In press.

Flegal, A. R., and J. H. Martin. 1977. Contamination of biological samples by ingested sediment. Mar. Poll. Bull. 8: 90-92.

Folsom, T. R., and D. R. Young. 1965. Silver-110m and cobalt-60 in oceanic and coastal organisms. Nature 206: 803-806.

Folsom, T. R., R. Grismore, and D. R. Young. 1970. Long-lived gamma-ray emitting nuclide silver-108m found in Pacific marine organisms and used for dating. Nature 227: 941-943.

Freeman, H. C., and D. A. Horne. 1973. Sampling the edible muscle of the swordfish (Xiphias gladius) for total mercury analysis. J. Fish. Res. Bd. Can. 30: 1251-1252.

Gaskin, D. E., K. Ishida, and R. Frank. 1972. Mercury in harbour porpoises (Phocoena phocoena) from the Bay of Fundy region. J. Fish. Res. Bd. Can. 29: 1644-1646.

Gilmartin, W. G., R. L. DeLong, A. W. Smith, J. C. Sweeney, B. W. De Lappe, R. W. Risebrough, L. A. Griner, M. D. Dailey, and D. B. Peakall. 1976. Premature parturition in the California sea lion. J. Wildlife Diseases 12: 104-115.

Hall, J. D., W. G. Gilmartin, and J. L. Mattsson. 1971. Investigation of a Pacific pilot whale stranding on San Clemente Island. J. Wildlife Dis. 7: 324-327.

Hatch, W. R., and W. L. Ott. 1968. Determination of sub-microgram quantities of mercury by atomic absorption spectrophotometry. Anal. Chem. 40: 2085-2087.

Hollander, M., and D. A. Wolfe. 1973. Nonparametric statistical methods. J. Wiley & Sons, New York. 503 pp.

Kerfoot, W. S., and A. S. Jacobs. 1976. Cadmium accrual in combined wastewater treatment-aquaculture system. Environ. Sci. Tech. 10: 662-667.

Koeman, J. H., W. S. M. van de Ven, J. J. M. de Goeij, P. S. Tjioe, and J. L. van Haaften. 1975. Mercury and selenium in marine mammals and birds. The Science of the Total Environmnet 3: 279-287.

Mackay, N. J., R. J. Williams, J. L. Kacprzac, M. N. Kazacos, A. J. Collins, and E. H. Auty. 1975. Heavy metals in cultivated oysters (Crassostrea commercialis = Saccostrea cucullata) from the estuaries of New South Wales. Austr. J. of Mar. and Freshwater Res. 26: 31-46.

Martin, J. H., and G. A. Knauer. 1973. The elemental composition of plankton. Geochim. Cosmochim. Acta 37: 1639-1653.

Martin, J. H., and W. W. Broenkow. 1975. Cadmium in plankton: Elevated concentrations off Baja California. Science 190: 884-885.

Martin, J. H., and A. R. Flegal. 1975. High copper concentrations in squid livers in association with elevated levels of silver, cadmium, and zinc. Mar. Biol. 30: 51-55.

Martin, J. H., P. D. Elliott, V. C. Anderlini, D. Girvin, S. A. Jacobs, R. W. Risebrough, R. L. DeLong, and W. G. Gilmartin. 1976a. Mercury-selenium-bromine imbalance in premature parturient California sea lions. Mar. Biol. 35: 91-104.

Martin, J. H., D. W. Bruland, and W. W. Broenkow. 1976b. Cadmium transport in the California Current, p. 159-184. In H. L. Windom, and R. A. Duce (eds.), Marine Pollutant Transfer, D. C. Heath and Co.

Martin, M., M. D. Stephenson, and J. H. Martin. 1977. Copper toxicity experiments in relation to abalone deaths observed in a power plant's cooling waters. Calif. Fish and Game 63: 95-100.

Nilsson, R. 1970. Aspects on the toxicity of cadmium and its compounds. Swedish Natl. Sci. Res. Council Ecol. Res. Comm. Bull. No. 7.

Odell, D. K. 1970. Premature pupping in the California sea lion, p. 185-190. In Proceedings of the 7th Annual Biosonar and Diving Mammal Conference, Stanford Research Institute, Menlo Park, California.

Parizek, J., I. Ostadalova, J. Kalouskova, A. Babicky, and J. Benes. 1971. The detoxifying effects of selenium: interrelations between compounds of selenium and certain metals, p. 85-122. In W. Merty, and W. E. Cornatzer (eds.), Newer Trace Elements in Nutrition, Marcel Dekker, Inc., New York.

Parsons, T., and M. Takahashi. 1973. Biological Oceanographic Processes. Pergamon Press, New York. 186 pp.

Patterson, C. C. 1965. Contaminated and natural lead environments of man. Arch. Environ. Health 11: 344-360.

Phillips, D. J. H. 1976. The common mussel, Mytilus edulis, as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals. Mar. Biol. 38: 59-69.

Phillips, D. J. H. 1977. Effects of salinity on the net uptake of zinc by the common mussel, Mytilus edulis. Mar. Biol. 41: 79-88.

Preston, A., D. F. Jeffries, J. W. R. Dutton, B. R. Harvey, and A. K. Steele. 1972. British Isles coastal waters: The concentrations of selected heavy metals in sea water, suspended matter, and biological indicators - a pilot survey. Environ. Poll. 3: 69-82.

Riley, J. P., and D. Taylor. 1972. The concentrations of cadmium, copper, iron, manganese, molybdenum, nickel, vanadium and zinc in part of the tropical north-east Atlantic Ocean. Deep-Sea Res. 19: 307-317.

Romeril, M. G. 1971. The uptake and distribution of Zn^{65} in oysters. Mar. Biol. 9: 347-354.

Schroeder, H. A., and J. Buckman. 1967. Reversal of cadmium hypertension in rats by a zinc chelate. Arch. Environ. Health 14: 693-698.

Schulze-Baldes, M. 1973. Die miesmuschel, Mytilus edulis, als indikator für die Bleikonzentration im Weserästuar und in der Deutschen Bucht. Mar. Biol. 21: 98-102.

Schutz, D. F., and K. K. Turekian. 1965. The investigation of the geographical and vertical distribution of several trace elements in sea water using neutron activiation analysis. Geochim. Cosmochim. Acta 29: 259-313.

Shigematsu, I., and Y. Hasegawa. 1971. Itai-Itai (ouch-ouch) disease and cadmium pollution in Japan, p. 49-64. In Conf. on Effects of Env. Trace Metals on Human Health, Honolulu.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman, San Francisco. 779 pp.

Soyer, J. 1963. Contribution à l'étude des effets biologiques du mercure et de l'argent dans l'eau de mer. Vie et Milieu 14: 1-36.

Trelease, S. F., and O. A. Beath. 1949. Selenium. Trelease and Beath, New York. 292 pp.

Underwood, E. J. 1971. Trace Elements in Human and Animal Nutrition. Academic Press, New York.

van Campen, D. R. 1966. Effects of zinc, cadmium, silver, and mercury on the absorption and distribution of copper-64 in rats. J. Nutrition 88: 125-130.

Vandevere, J. E. 1969. Feeding behavior of the southern sea otter, p. 87-94. In Proc. Sixth Ann. Conf. Biol. Sonar and Div. Mamm., Stanford Research Institute, Menlo Park, California.

Vedros, N. A., A. W. Smith, J. Schonewald, G. Migaki, and R. C. Hubbard. 1971. Leptospirosis epizootic among California sea lions. Science 172: 1250-1251.

Windom, H. L., and R. G. Smith. 1972. Distribution of iron, magnesium, copper, zinc, and silver in oysters along the Georgia coast. J. Fish. Res. Bd. Can. 29: 450-452.

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Environmental Research Information Center
Cincinnati, Ohio 45268

OFFICIAL BUSINESS
PENALTY FOR PRIVATE USE $300
AN EQUAL OPPORTUNITY EMPLOYER

POSTAGE AND FEES PAID
U.S. ENVIRONMENTAL PROTECTION AGENCY
EPA-335

Special Fourth-Class Rate
Book

If your address is incorrect, please change on the above label tear off; and return to the above address.
If you do not desire to continue receiving these technical reports, CHECK HERE ☐; tear off label, and return it to the above address.

EPA-600/3-79-038

11
2052